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Action of progesterone receptor modulators on uterine leiomyomas

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Summary
Novel progesterone receptor modulators (PRMs) have recently been demonstrated to be effective in the treatment of patients with symptomatic uterine leiomyomata. PRMs are shown to reduce leiomyoma size and improve leiomyoma-associated symptoms. However, the precise mechanisms underlying the action of PRMs remain to be elucidated. My co-workers and I have investigated in vitro action of PRMs in cultured leiomyoma cells and revealed that PRMs inhibit cell proliferation and induce apoptosis of leiomyoma cells. Moreover, our recent studies show that PRMs can modulate the metabolism of extracellular matrix proteins in cultured leiomyoma cells toward the collagenolysis. The update about an action of PRMs in uterine leiomyoma cells in vitro is described in this article.

Key words: Progesterone receptor modulator; Leiomyoma; Proliferation; Apoptosis; Angiogenesis; Extracellular matrix.

Traditionally, it has been thought that estrogen mainly acts to promote the growth of uterine leiomyomas. However, recent investigations have accumulated the evidence that progesterone also promotes the growth of uterine leiomyoma cells through a cross-talk with growth factors [1]. In this context, attention has been paid to the application of progesterone receptor modulators (PRMs) in the treatment of uterine leiomyomas.

Two recent studies have demonstrated that novel PRMs, asoprisnil and CDB-2914, are effective in the treatment of symptomatic uterine leiomyomas [2, 3]. Asoprisnil was shown to suppress uterine bleeding in patients with leiomyomas, and reduces leiomyoma and uterus volume [2]. The median percentage changes in leiomyoma size and uterus volume from baseline were reported to be 36% and 17% at week 12 in patients receiving a 25-mg dose of asoprisnil [2]. Similarly, three-month treatment with 10 mg and 20 mg dosages of CDB-2914 has been reported to result in a respective 36% and 21% reduction in leiomyoma volume [3]. PRMs did not cause hypooestrogenism, suggesting minimal adverse effects on bone mineral density. The safety of these drugs was confirmed in clinical trials, but a single case of endometrial cystic hyperplasia developed in a patient receiving CDB-2914 [3]. The optimal treatment regimen for PRMs remains undetermined, and the effects of long-term use of PRMs on safety and efficacy outcomes should be assessed [2].

However, the precise mechanisms underlying the action of PRMs remain to be elucidated. To explore the mechanisms by which PRMs reduce the size of uterine leiomyomas, we have investigated the effects of PRMs, asoprisnil and CDB-2914, on the growth, apoptosis, growth factor expression, and metabolism of the extracellular matrix proteins in cultured leiomyoma cells. The diverse actions of PRMs on cultured leiomyoma cells can be summarized as follows:

1) PRMs inhibit cell proliferation of cultured leiomyoma cells [4, 5].
2) PRM suppresses the protein contents of growth factors in cultured leiomyoma cells, including epidermal growth factor, insulin-like growth factor-I, and transforming growth factor β3, and their receptors [6]. PRM antagonizes the growth stimulatory action of growth factors in cultured leiomyoma cells.
3) PRM suppresses the protein contents of angiogenic factors and their receptors in cultured leiomyoma cells, including vascular endothelial growth factor and adrenomedullin [7].
4) PRMs induce apoptosis of cultured leiomyoma cells through activation of the mitochondrial pathway and tumor necrosis factor-related apoptosis-inducing ligand-mediated apoptotic pathway [4, 5, 8]. PRM increases the protein contents of proapoptotic proteins such as Bax and Bak, but decreases the protein content of antiapoptotic protein such as Bcl-2 [4, 5, 9].
5) PRM elicits endoplasmic reticulum stress in cultured leiomyoma cells, leading to the induction of apoptosis [9]. PRM augments the expression of the proapoptotic proteins such as growth-arrest- and DNA-damage-inducible gene 153 (Gadd153) and tribbles-related protein 3 in cultured leiomyoma cells. RNA interference of Gadd153 confirmed the close association of Gadd153 in the induction of apoptosis in cultured leiomyoma cells.
6) PRMs promote collagen degradation in cultured leiomyoma cells by modulating the expression of several extracellular matrix proteins [10, 11]. PRMs augmented the expression of extracellular matrix metalloproteinase inducer (EMMPRIN) and metalloproteinases (MMPs), but attenuated the expression of tissue inhibitors of MMP (TIMPs) and type I and III collagen protein levels in cultured leiomyoma cells. RNA interference of EMMPRIN abrogates both PRM-mediated induction of MMPs and reduction of TIMPs and collagens in cultured leiomyoma cells.

In these serial studies, it is noteworthy that PRMs did not affect the growth, apoptosis, growth factor expression, and extracellular matrix metabolism in cultured myometrial cells. This suggests that PRMs may not inversely affect the growth of myometrial cells.

PRMs were shown to inhibit the growth of cultured leiomyoma cells, suggesting that PRMs exhibit progesterone antagonist activity on cultured leiomyoma cells. Although increasing data indicate the promoting activity of progesterone on leiomyoma growth [1], there is little information regarding progesterone-regulated genes in uterine leiomyomas. It is speculated that the unknown mechanisms by which PRMs induce growth suppression of leiomyoma cells are involved. Microarray studies will reveal the genes regulated by PRMs in cultured leiomyoma cells. This elucidation would contribute to the understanding of more detailed mechanisms responsible for PRM-regulated inhibition of leiomyoma growth.

In conclusion, recent clinical data demonstrate that PRMs are effective even in the short-term treatment of patients with symptomatic uterine leiomyomas. Our in vitro data corroborated the growth inhibitory action of PRMs in cultured leiomyoma cells in the absence of comparable effects on myometrial cells. The elucidation of the precise mechanisms by which PRMs regulate the growth of uterine leiomyomas will shed new light on the clinical application of these novel drugs in the treatment of uterine leiomyomas.

Acknowledgement

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References


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Improved pregnancy outcome for women with decreased ovarian oocyte reserve and advanced reproductive age by performing in vitro fertilization-embryo transfer

The University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School at Camden, Cooper Hospital/University Medical Center, Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology & Infertility, Camden, NJ (USA)

Summary

Objective: To compare the pregnancy rates with IVF-ET vs non-assisted reproductive technology in women of more advanced reproductive age with decreased egg reserve as manifested by elevated day 3 serum FSH. Methods: A retrospective evaluation was made in women aged ≥ 38 with a day 3 serum FSH of ≥ 15 mIU/ml with ≥ 1 year of infertility. Another inclusion criterion was three cycles (unless a pregnancy occurred before that time) of either IVF-ET or non-assisted reproductive therapy which included luteal phase support with progesterone. Results: The clinical pregnancy rates in three cycles for non-IVF were 11.7% vs 27.2% for IVF. Delivery rates were 2.9% vs 15.1%. For ages 40-42 the clinical pregnancy rates were 37.5% vs 0.0% (p = .02). Conclusions: Live deliveries are possible in women ≥ age 38 with marked decreased egg reserve. In vitro fertilization is more effective than non-IVF when follicle stimulation with gonadotropins is mild.

Key words: Diminished egg reserve; In vitro fertilization; Minimal stimulation, FSH.

Introduction

Many factors affect fertility in the general population. Two factors limiting fertility include advanced maternal age and poor ovarian reserve, as shown by elevated day 2 or 3 serum follicle stimulating hormone (FSH) levels. There is a decline in fecundity that accelerates between 35 and 40 years of age and approaches zero by age 45 [1]. As one ages, or as menopause approaches, there is a paucity of ovarian follicles [2]. In aging women, a natural selection process has occurred at each previous cycle, whereby the oocytes recruited during each cycle were the ones with the best quality. The remaining follicles are of lower caliber – having extra chromosomes and defective cytoplasmic mitochondria [3]. Some studies have concluded that an elevated FSH level at any age decreases pregnancy rates in assisted reproductive technologies (ART) [4]. However, in the younger patient with elevated serum FSH levels, it has not been determined whether the oocytes undergo an accelerated atretic process or another indiscriminatory destructive process in which both the “best” and “worst” immature oocytes are destroyed [5]. Regardless of the mechanism, elevated serum FSH levels and advanced age provide a ‘double hit’ to fertility.

Despite the above discouraging assessments, several case reports have established precedents that pregnancy remains possible given advanced age and imminent ovarian failure [6-11]. Early studies of euestrogenic women with elevated serum FSH levels have shown that without in vitro fertilization (IVF) or gamete intrafallopian transfer (GIFT), pregnancy rates varied based on age [5]. Previous studies have also found a much worse prognosis for women with elevated day 3 serum FSH levels in the age range of 38-45 in both IVF cycles and non-IVF cycles compared to younger women with elevated FSH [5, 12]. Thus one might conclude that the combination of advanced age and elevated day 3 FSH levels leads to low pregnancy outcomes. Incorrectly, donor egg programs are recommended to these women as the only means of achieving pregnancy. Recent data have shown that pregnancy rates per embryo transfer (ET) vary from 21.7% in women aged 40-42 and up to 30.8% in women aged 36-39 [13] following single ET despite decreased oocyte reserve [13]. Thus, pregnancy with one’s own eggs remains a viable option for older women experiencing ovarian failure.

Women older than age 40 elect either IVF or non-IVF to achieve a pregnancy. In vitro fertilization is often chosen if there are tubal factors and/or severe male factor. Women with patent fallopian tubes and/or mild male factor problems have the option of using either IVF or non-IVF. However, one of the pitfalls of IVF is the decline in success rate with increasing maternal age. One study showed that in older patients, GIFT has a higher success rate than IVF [14]. Several studies have alluded to the role of the fallopian tube in improving fertilization and embryo development [15].

The function of the fallopian tube includes: ovum pickup, sperm transport, ovum and sperm capacitation, ovum fertilization, zygote development and transfer [15]. One other function of the fallopian tube is to allow hatch-
ing of the embryo out of the embryo membrane by providing certain enzymes [16, 17]. Though there are data that provide information on assisted embryo hatching with acidic Tyrode’s solution or laser drilling showing improved success rates in older women having fresh ET or women of all ages having frozen ET, the possibility exists that the efficiency of these artificial procedures is inferior to natural hatching as the embryo traverses the fallopian tubes [16, 17].

To date there have only been retrospective studies comparing efficacy of IVF with GIFT [18]. It has not yet been established whether one technique is superior to the other, and under what conditions. Cost considerations suggest that non-IVF treatments should be used initially if possible (patent fallopian tubes and adequate sperm). Early comparative studies of GIFT vs IVF found that possibly the former may be superior to the latter for the older reproductive group [18]. Thus, the possibility exists that maybe for this advanced age group, who with decreased egg reserve will not produce very many embryos with IVF, the advantage of the embryos traversing the fallopian tube if non-IVF methods are employed could obviate the usual advantage of IVF of multiple embryos and provide equal or even superior pregnancy rates.

The study presented herein attempts to determine if IVF-ET results in a higher pregnancy rate in this older group with elevated day 3 serum FSH vs correcting follicular maturation, sperm-mucus interaction problems, and correcting luteal phase defects without IVF.

Materials and Methods

Female patients who presented during a seven year period, from 1996 to 2003, who met the following criteria were included in the study: FSH ≥ 15 mIU/ml, age ≥ 38, and infertility (primary or secondary) > 1 year. Each woman was evaluated as to pregnancy outcome (clinical or ongoing/delivered pregnancy past the first trimester). The pregnancy outcome was determined for the first three cycles of treatment.

A total of 67 female subjects were included in the study. The women were stratified into two groups depending on whether IVF-ET was performed or not.

All patients were treated in the luteal phase with at least 200 mg progesterone vaginal suppositories twice daily to compensate for the greater uterine need for progesterone at an older age. The dosage of progesterone was increased by IM injection if the woman did not attain a homogeneous hyperechogenic pattern in the mid-luteal phase. A non-homogeneous hyperechogenic pattern on ultrasound indicates lower fertility potential [19, 20].

Follicular maturation issues were resolved by using minimal stimulation with gonadotropins. Non-IVF cases were not given any follicle maturation if they attained a mature follicle ≥ 17 mm by sonography with a serum estradiol (E2) level of at least 175 pg/ml. Failing to achieve a mature follicle resulted in the minimal use of gonadotropins, usually 75 IU once the serum E2 was ≥ 100 pg/ml.

For IVF cases no more than 75-150 IU of gonadotropins were used from the earliest day 3. They were never given while the serum FSH was increased ≥ 11 mIU/ml. Frequently they were only given when a follicle advanced far enough to secrete sufficient estradiol to lower the FSH. The various follicular stimulation methods have been previously described in detail [21].

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### Table 1. — Clinical pregnancy and delivery rates following non-IVF and IVF treatment.

<table>
<thead>
<tr>
<th></th>
<th>Non-IVF Group</th>
<th>IVF Group</th>
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<tr>
<td>Number</td>
<td>34</td>
<td>33</td>
</tr>
<tr>
<td>Average age</td>
<td>41.0 ± 2.3</td>
<td>40.7 ± 2.0</td>
</tr>
<tr>
<td>Average serum FSH (mIU/ml)</td>
<td>29.6 ± 13.0</td>
<td>22.6 ± 7.4</td>
</tr>
<tr>
<td>No. of clinical pregnancies</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>No. of deliveries</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Delivery rate/patient</td>
<td>2.9% (1/34)</td>
<td>15.1% (5/33)</td>
</tr>
<tr>
<td>Clinical pregnancy rate per patient</td>
<td>11.7% (4/34)</td>
<td>27.2% (9/33)</td>
</tr>
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</table>

### Table 2. — Clinical pregnancy rates after three cycles of treatment for patients aged 38-39, 40-42 and 43-45.

<table>
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<th>Age 38-39 years</th>
<th>Age 40-42 years</th>
<th>Age 43-45 years</th>
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</thead>
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<tr>
<td>IVF (n = 33)</td>
<td>30% (3/10)</td>
<td>37.5% (6/16)</td>
</tr>
<tr>
<td>Non-IVF (n = 34)</td>
<td>9.1% (1/11)</td>
<td>0.0% (0/13)</td>
</tr>
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</table>

p-value, Fisher’s exact test 0.311 0.02

Cases with male factor not using IVF were treated with intrauterine insemination (IUI). Intracytoplasmic sperm injection (ICSI) was performed for male factor when IVF was performed. For patients undergoing IVF, embryos were transferred on day 3 and assisted hatching was performed prior to transfer. If the egg of a woman intending to do IVF released before retrieval, an IUI would be performed. However once designated as IVF, the cycle would not count as a non-IVF cycle but not be included as an IVF cycle either.

Only women having three treatment cycles (IVF or non-IVF) were included unless a pregnancy occurred first (only one pregnancy was allowed even if it was a miscarriage). Fisher’s exact test was used to compare delivered pregnancy rates between non-IVF and IVF groups.

### Results

There were 34 non-IVF patients with an average age of 41.0 ± 2.3 years versus 33 IVF patients with an average age of 40.7 ± 2.0 years. Using the highest baseline FSH recorded the mean serum FSH was 29.6 ± 13.0 mIU/ml for non-IVF cases and 22.6 ± 7.4 for IVF cases.

A comparison between hypergonadotropic females treated without or with IVF-ET is shown in Table 1. There were four clinical pregnancies (11.7%) in the non-IVF group versus nine (27.2%) in the IVF group. The delivery rates per cycle were 2.9% (1/34) for non-IVF versus 15.1% (5/33) for IVF. The difference in delivered pregnancies did not quite reach significance (p = .092, Fisher’s exact test). Three of four non-IVF pregnancies miscarried. The IVF group had three miscarriages and one ectopic pregnancy.

Analysis of the data based on different age ranges of 38-39, 40-42 and 43-45 is shown in Table 2. Fisher’s exact test p value was significant for women aged 40-42 years. In vitro fertilization treatment for these women resulted in a 37.5% clinical pregnancy rate compared to 0% for the non-IVF treatment in three treatment cycles.

### Discussion

These data provide information on pregnancy outcome in the largest series to date of women of advanced reproductive age and hypergonadotropism. The 2.9% viable
pregnancy rate in three cycles in this group of women was similar to previous findings of 5.5% in six cycles [5]. Thus live deliveries with and without IVF are possible in women with decreased egg reserve as manifested by day 3 serum FSH, levels > 15 mIU/ml even in women aged ≥ 40. In vitro fertilization with embryo transfer seems to improve the likelihood of success in this most difficult group.

A previous study has shown reasonable pregnancy rates for women aged 40-42 years old and elevated serum FSH with a delivery rate per transfer of 21.7%, and 0% for women aged > 43 years old [13]. In this study when women were stratified based on age, there was a significant difference in pregnancy rates between the two treatment modalities. It is possible that the pregnancy rates of both the non-IVF and IVF group were skewed by the inclusion of women > 43 years old (Table 2). In fact by eliminating the 43-45 age group the clinical pregnancy rate per three cycles was 9/26 (34.2%) with IVF vs 3/24 (12.5%) with non-IVF (p < .05, Fisher’s exact test). The viable delivery rate for women aged 38 to 42 years was 5/26 (19.2%) with IVF vs 1/24 (4.2%) with non-IVF. The chances of completing a viable pregnancy and delivery increase five-fold with the use of IVF. Although there was no statistically significant difference between IVF and non-IVF, a five-fold raw increase in viable pregnancies may be further delineated with a larger sample size.

Several other methods have been proposed to increase the pregnancy rates among older women with elevated FSH levels. The use of donor oocytes would increase the pregnancy success rate of infertile couples, however such an option may not be a consideration in couples who for religious, financial and personal reasons would never consider the donor oocyte program.

The aim of this report was to assess how successful IVF procedures are in securing a live birth in older women with decreased ovarian reserve. Statistical significance was reached with the Fisher exact test (p = 0.02) when comparing women aged 38-39 versus women aged 40-42. The study reinforces the need for aggressive treatment in older women with decreased ovarian reserve. Non-IVF treatments, while often preferred in the younger patient, would serve to only prolong infertility among women with advanced reproductive age. Such data grant a couple more information to provide a basis for either choosing to proceed with infertility treatments using their own eggs or deciding to use donor eggs. In vitro fertilization-embryo transfer provides a higher pregnancy rate per cycle but obviously at an increased expense. However, one does not have to be concerned with the biggest risk of IVF which is ovarian hyperstimulation.

References


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Improved pregnancy outcome for women with decreased ovarian oocyte reserve and advanced reproductive age by performing etc.
Normal pregnancy resulting from a non-pronuclear oocyte at the time of examination for fertilization

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Summary

Purpose: To report the case of a patient undergoing in vitro fertilization (IVF) in which a non-pronuclear (0PN) oocyte resulted in a normal pregnancy. Methods: A 36-year-old woman underwent an IVF-embryo transfer treatment cycle. Results: Four oocytes were retrieved for insemination by IVF. Examination for fertilization revealed two polypronuclear polygynic and two non-pronuclear oocytes. The non-pronuclear oocytes were observed further for development. One embryo developed from the non-pronuclear cohort and was transferred at the 8-cell stage on day 3. Subsequently, a pregnancy developed, and resulted in the delivery of a healthy term infant. Conclusions: Non-pronuclear oocytes may represent a source of developmentally competent embryos, and further observation of this cohort should be considered, particularly in situations involving a low yield of oocytes at retrieval.

Key words: Fertilization; Infertility; Non-pronuclear.

Introduction

During the process of in vitro fertilization, the assessment of fertilization is typically performed from 16 to 20 hours after insemination or intracytoplasmic sperm injection [1]. Normal fertilization is characterized by the presence of two pronuclei, and these zygotes are observed further for cleavage.

The finding of non-pronuclear (0PN) oocytes at the time of examination for fertilization is not uncommon. In one series, the incidence of 0PN oocytes after intracytoplasmic sperm injection (ICSI) was reportedly 24% [2]. The approach to non-pronuclear oocytes among IVF centers is variable. While some adhere to a policy of continued observation of these oocytes, other centers discard them after the evaluation. We present a case of a normal pregnancy resulting from an oocyte which was non-pronuclear at the time of fertilization evaluation.

Case Report

A 36-year-old Caucasian nulligravida presented with a two-year history of infertility, refractory to three cycles of clomiphene citrate and two cycles of IVF. Hormonal evaluation revealed a day 3 FSH of 5.0 mIU/ml and estradiol of 29 pg/ml. A clomiphene citrate challenge test was performed with a day 10 FSH of 8.6. The patient’s history was notable for hyperprolactinemia, for which she was maintained on bromocriptine, 2.5 mg daily, with subsequent normalization of the serum prolactin level. The hysterosalpingogram was normal. Her husband’s medical history was unremarkable, and his semen analysis was within normal limits.

The patient proceeded with a third IVF treatment cycle. Ovarian stimulation was conducted with 450 IU/day for eight days of recombinant FSH and human menopausal gonadotropins. Once daily antagonist (ganirelix) injection was initiated when the lead follicle reached a mean diameter of 14 mm. At retrieval timed 35 hours after human chorionic gonadotropin (hCG) injection, four metaphase II oocytes were aspirated from five mature follicles. These were inseminated with 100,000 motile spermatozoa per ml and cultured in IVF medium. At fertilization evaluation 20 hours post insemination, two polypronuclear polygynic oocytes and two non-pronuclear oocytes were documented. Our laboratory policy is to discard digynic/polypronuclear polygynic oocytes and to monitor non-pronuclear oocytes for cleavage potential. On day 2 post retrieval, a four-cell embryo was observed. On day 3, the embryo had developed to the 8-cell stage and assisted hatching was conducted prior to uterine transfer. The patient conceived with a single intrauterine pregnancy confirmed by ultrasound at six weeks of gestational age. She proceeded to deliver a healthy, 3,690 g female infant at 40 weeks of gestation.

Discussion

The series of events that occurs after fertilization of the oocyte are well described. After fertilization, decondensation of the sperm head is followed by extrusion of the second polar body and appearance of the pronuclei. Time-lapse recording of events involved in pronuclear formation after ICSI demonstrate that only 63% of oocytes evidence simultaneous appearance of male and female pronuclei [2]. Asynchrony of pronuclear formation presumably occurs secondary to accelerated formation of either male or female pronuclei. Asynchrony is more frequently observed in IVF than in ICSI [3]. The process of syngamy involves the breakdown of pronuclear membranes and the reorganization of maternal and...
Normal pregnancy resulting from a non-pronuclear oocyte at the time of examination for fertilization

paternal chromosomes to form a zygote. In contrast to their formation, the disappearance of pronuclei is nearly always synchronous. It is possible that in cases of non-pronuclear oocytes, the assessment may be timed such that the pronuclei are missed due to their collectively early or late formation and disappearance.

The finding of 0PN oocytes at the time of fertilization evaluation may be considered evidence of failed fertilization. Yet, the validity of this conclusion is based on accurate identification of organelles and on the allowance of adequate time for the assessment of the pronuclei. Pronuclear formation can be seen as early as six hours post insemination and as late as 20 hours [1]. The concurrent finding of polypronuclearpolygynic oocytes with the 0PN oocytes at the time of the fertilization check is significant, suggesting that the window for visualization of pronuclei was not missed.

We conducted a survey of IVF centers in our region. Three of seven (42%) centers reported discarding 0PN oocytes. The remaining centers reported continued observation of these oocytes for cleavage potential. The developmental potential of fertilized oocytes with less than two pronuclei at the time of fertilization check is evidenced by our case.

An extensive search of the international literature on this subject (PubMed, Ovid, Science Direct) shows that this is the first case report of term delivery of a healthy female infant after the transfer of a cleavage stage embryo derived from a non-pronuclear oocyte at fertilization check. We report transfer of a cleavage stage embryo derived from a non-pronuclear oocyte at fertilization check, which resulted in the term delivery of a healthy female infant. As described for 1PN oocytes, the finding of 0PN oocytes at fertilization check is not necessarily indicative of their developmental capacity. Our survey of IVF center practices indicates that monitoring of non-pronuclear oocytes is not universal. The possibility for embryo development from non-pronuclear oocytes may provide an under-recognized source of embryos for subsequent transfer. Surveillance of these oocytes beyond the fertilization check is therefore advisable, particularly for the couple with a low number of oocytes at retrieval.

References

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Inherited thrombophilia screening in Greek women with recurrent fetal loss

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Summary

Objective: The present study was designed to determine the prevalence of factor V Leiden (FVL), prothrombin gene G20210A (PTG) and methylenetetrahydrofolate reductase (MTHFR C677T) mutations in women from South-Western Greece with recurrent fetal loss (RFL) and negative personal thromboembolic history. Materials and Methods: 212 women with RFL and 181 women with at least two pregnancies with normal outcome and no history of pregnancy loss were investigated for the commonest thrombophilic mutations (FVL, PTG, MTHFR C677T). Comparisons between groups were performed by Pearson’s chi-square test and odd ratios were calculated. Results: An abnormal genotype was detected in 49 women of the study group (23.1%) and in 41 women of the control group (22.6%). Conclusion: Inherited thrombophilia screening is not indicated as an initial approach in Greek women with RFL and negative personal thromboembolic history.

Key words: First trimester recurrent fetal loss; Factor V Leiden; Prothrombin G20210A; MTHFR C677T; Molecular thrombophilic testing.

Introduction

Early fetal loss is separated into recurrent fetal loss in the 1st trimester and single fetal loss in the 2nd trimester [1]. First trimester recurrent fetal loss (RFL) has been defined as three or more consecutive pregnancy losses before the 12th week of gestation, affecting 1% of the women in reproductive age [2].

Several factors have been reported as causes of RFL. These include chromosomal abnormalities, uterine structural abnormalities, infections, metabolic disorders, autoimmune factors, drugs and environmental factors [3].

The present study was designed to determine the prevalence of factor V Leiden (FVL), prothrombin gene G20210A (PTG) and methylenetetrahydrofolate reductase (MTHFR C677T) mutations in women from South-Western Greece with RFL and negative personal thromboembolic history.

Materials and Methods

From October 1999 to December 2006, 212 women with a history of RFL attending the Outpatient Clinic of the Obstetrics and Gynecology Department of the University of Patras, were included in the study. A control group consisted of 181 age- and race-matched women, with at least two pregnancies with normal outcome and no history of pregnancy loss. Demographic data were collected during the first appointment at the Clinic and included information on thromboembolic and obstetric history, current medications, and family history.

Exclusion criteria for entry in the study were known risk factors for RFL. These included inherited thrombophilic factors (FVL, PTG, homozygous MTHFR C677T mutations as well as protein C, protein S and antithrombin III deficiencies), personal thromboembolic history, preexisting antiphospholipid syndrome (APS), chromosomal abnormalities, uterine structural abnormalities, hormonal imbalances, infections, metabolic disorders, autoimmune disorders and drugs. The study was approved by the Ethical Committee of the Hospital. Informed consent was obtained from each woman.

Blood samples in EDTA were collected from all women. Molecular diagnosis of the FVL, PTG, homozygous MTHFR C677T mutations was performed after DNA isolation, polymerase chain reaction (PCR) amplification, and hybridization of amplification products using allele specific oligonucleotide probes for the detection of factor V G506A, prothrombin G20210A and MTHFR C677T normal and mutated alleles (Thrombophilia Gene Mutation Assay Kit, Vienna Lab, Austria).

Comparisons between groups were performed by Pearson’s chi-square test. Odds ratio (OR) and 95% confidence intervals (CI) were calculated. The significance level was set at 0.05. Statistical analyses were performed by the Statistical Package for Social Sciences for Windows (SPSS-12).

Results

An abnormal genotype was detected in a total of 49 of the 212 women in the study group (23.1%). Among them, eight women (3.8%) were heterozygous for the FVL mutation (FVL (±) genotype), eight (3.8%) were heterozygous for the PTG mutation (PTG (±) genotype) and 33 women (15.6%) were homozygous for the MTHFR C677T mutation (MTHFR T677T genotype). Combined thrombophilic genotypes or homozygosity for the FVL mutation and PTG mutation were not detected.

An abnormal genotype was detected in a total of 41 from the 181 women in the control group (22.6%). Among them, seven women (3.9%) were heterozygous for the FVL mutation (FVL (±) genotype), seven (3.9%)
were heterozygous for the PTG mutation (PTG (±) genotype) and 27 women (14.9%) were homozygous for the MTHFR C677T mutation (MTHFR T677T genotype). Combined thrombophilic genotypes or homozygosity for the FVL mutation and PTG mutation were not detected.

The ORs for the incidence of thrombophilic mutations in the women with RFL as compared with the control group are shown in Table 1.

Table 1.— Prevalence of thrombophilic genotypes among women with RFL and control women.

<table>
<thead>
<tr>
<th>Thrombophilic genotype</th>
<th>Women with RFL (n = 212)</th>
<th>Control women (n = 181)</th>
<th>OR (± 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVL (±)</td>
<td>8 (3.8%)</td>
<td>7 (3.9%)</td>
<td>0.97 (0.35-2.74)</td>
</tr>
<tr>
<td>PTG (±)</td>
<td>8 (3.8%)</td>
<td>7 (3.9%)</td>
<td>0.97 (0.35-2.74)</td>
</tr>
<tr>
<td>MTHFR T677T</td>
<td>33 (15.6%)</td>
<td>27 (14.9%)</td>
<td>1.05 (0.61-1.87)</td>
</tr>
<tr>
<td>Total</td>
<td>49 (23.1%)</td>
<td>41 (22.6%)</td>
<td>1.03 (0.64-1.65)</td>
</tr>
</tbody>
</table>

(±) = heterozygosity; OR = Odds Ratio; CI = Confidence Interval.

Discussion

Successful pregnancy outcome depended on the development and maintenance of adequate placental circulation. Abnormalities of placental vasculature may result in a number of gestational pathologies, including 1st and 2nd trimester fetal loss, intrauterine growth restriction (IUGR), intrauterine fetal death (IUFD), placental abruption (PA) and preeclampsia (PE) [4].

The pathogenetic mechanisms responsible for placental vascular pathologies in women with thrombophilia have not been fully elucidated. It is yet unknown why only some women with thrombophilia express vascular gestational pathologies while others do not. It is possible that this may relate to local factors affecting coagulation, fibrinolysis, and vascular tone at the level of placental vessels [5].

Many studies confirmed that women with inherited thrombophilia are at increased risk of developing pregnancy complications [1, 6]. In our study, we investigated the association between inherited thrombophilia and RFL in women from South-Western Greece with a negative personal thromboembolic history.

The presence of FVL (±) genotype in our study showed a negative association with RFL, but this finding was not significant (OR 0.97; 95% CI 0.35-2.74). According to the international literature the presence of FVL (±) genotype increase the risk for RFL (OR 1.91; 95% CI 1.01-3.61), compared to a normal genotype [6-13].

The presence of PTG (±) genotype in our study showed a negative association with RFL, but this finding was not significant (OR 0.97; 95% CI 0.35-2.74). According to the international literature the presence of PTG (±) genotype increase the risk for RFL (OR 2.70; 95% CI 1.37-5.35), compared to a normal genotype [6-8, 11, 12, 14-16].

The presence of MTHFR T677T genotype in our study showed a positive association with RFL, but this finding was not significant (OR 1.05; 95% CI 0.61-1.87).

According to the international literature the presence of MTHFR T677T genotype showed a negative association with RFL, but this finding was not significant (OR 0.86; 95% CI 0.44 to 1.69) [6, 11, 17, 18].

The findings from many studies have shown that selective inherited thrombophilia screening in women with personal thromboembolic history and/or adverse pregnancy outcome is more cost-effective than universal screening [6, 19, 20]. As these complications tend to recur in subsequent pregnancies, the identification of inherited thrombophilia markers in these patients is necessary. It offers an opportunity to reduce the risk of recurrence with prophylactic anticoagulant therapy [21-23].

Although many studies confirmed that women with inherited thrombophilia are at increased risk of developing pregnancy complications [1, 6], the absolute risk of venous thromboembolic disease and adverse pregnancy outcome remains low. Thus, at present, universal inherited thrombophilia screening in women during pregnancy cannot be justified clinically [1, 6].

The main limitation of our study was the small number of cases. Our results suggest that, inherited thrombophilia screening is not indicated as an initial approach in Greek women with RFL and negative personal thromboembolic history. These data require confirmation in larger clinical trials.

Conclusion

Our study suggests that inherited thrombophilia screening is not indicated as an initial approach in Greek women with RFL and negative personal thromboembolic history.

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HPV detection and genotyping as an earlier approach in cervical cancer screening of the female genital tract

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Summary

Human papillomavirus (HPV) infection is the leading risk factor for cervical intraepithelial neoplasia (CIN) and cervical cancer. More than 100 virus genotypes have been identified so far, some of them strongly associated with the development of neoplasia. The aim of this study was to evaluate the prevalence of the different HPV genotypes in women presenting no cytological alterations in cervical cells, in women presenting light alterations, and in women presenting severe alterations at routine gynecological examination. We retrospectively analyzed 97 HPV results of women submitted to cervical cancer screening compared to their Papanicolaou and colposcopy examinations. Data were analyzed individually and within groups to correlate the HPV genotypes identified by polymerase chain reaction (PCR) and the respective alterations in cervical cells. Among the nine cases diagnosed as CIN I (9.3%), two were positive for low-risk HPV genotypes (22%), and the other seven were negative for HPV by PCR (78%). CIN II or CIN III diagnoses were associated with positive HPV results by PCR in four cases (36%), for high-risk as well as low-risk genotypes. There were two patients with severe cytological alterations in cervical cells, but with an indeterminate HPV genotype (18%), and one case with a negative HPV result (9%). Among the 57 cases without cytological alterations, seven were positive for low-risk HPV genotypes (12%) and two for high-risk HPV genotypes (3.5%). In the 48 remaining cases, we observed one with an indeterminate HPV genotype (22%), and the other 47 were negative for HPV by PCR (47%). Our study demonstrates an important prevalence of high- and low-risk genotypes in our population, including those not present in the commercially available vaccine, even in patients with no evidence of cytological alterations in cervical cells. These results highlight the usefulness of HPV detection and typing as an early approach for cervical cancer screening and prevention.

Key words: HPV; Genotypes; CIN; Papanicolaou; PCR, cancer.

Introduction

The human papillomavirus (HPV) is an oncogenic microorganism which occurs naturally in humans, inducing epithelial proliferation during the course of a productive infection, and is known as being constantly associated with cervical cancer [1]. HPV invades germinative epithelial cells through micro lesions, and the resulting infection may be transient or persistent [2, 3]. Even if infection with oncogenic HPV genotypes is frequent among sexually active women, most cases are self-limiting [4]. Development of cervical lesions occurs only in a small proportion of women who present persistent infection with oncogenic genotypes [5, 6]. Integration of the virus genome in malignant cells has been demonstrated in every case of cervical cancer, what is believed to be a necessary condition for the development of neoplasia [7].

HPV actually comprises a heterogeneous group of viruses with more than 100 different genotypes, from which about 40 are capable of infecting the anogenital region. Among the anogenital genotypes, the 16, 18, 31, 33, 35, 45, 51, 52, 56, 58, 59, 68, 73 and 82 are classified as high risk for development of neoplasia, for being clearly identified in patients with malignant lesions [5, 8].

Uterine cervical cancer is the second most frequent neoplasia in women, but in Latin America, prevalence rates may be about four times higher than those found in developed countries [9, 10]. Despite the fact that, in our country, data about the prevalence of different HPV genotypes are abundant [11, 12], in certain locations awareness about the importance of HPV detection in the prevention of cervical cancer is still scarce, such as the prevalence of HPV in women presenting light cytological alterations (CIN I, ASCUS or AGUS), and also the importance of monitoring HPV infection after therapeutic measures for severe alterations.

The simplest method for prevention of cervical cancer is the Papanicolaou smear or liquid cytology test, considered as an examination of excellence in evaluating the degree of cellular alterations in squamous cervical epithelium, that has contributed to drastically reduce the incidence of cervical cancer around the world. However, in the last decades, several studies have pointed out non-ideal rates of sensitivity of the conventional smear preparation, what may vary from 50% to 60% [13].

Today, two methods are widely used for HPV detection: Hybrid Capture (HC) and the polymerase chain reaction (PCR) with consensus primers. PCR is virtually capable of detecting every anogenital HPV genotype with greater sensitivity, also available in the form of standardized commercial kits [14]. The Hybrid Capture II test (HC II) is capable of detecting the DNA of 18 HPV genotypes among those commonly infecting the anogenital region (of males and females), and a positive result is reported as presence of high- (A) or low-risk (B) HPV [15].
With this picture in mind, we have aimed to evaluate the prevalence of the different HPV genotypes in women undergoing cervical cancer screening in our population. We have also aimed to determine the prevalence of these genotypes in women presenting no cytological alterations in cervical cells, in those presenting light alterations, and in women already presenting severe alterations, to evaluate the feasibility of HPV detection and typing as an early approach in cervical cancer screening and prevention.

Materials and Methods

We have retrospectively evaluated the results of HPV detection and typing in the cervical samples of 97 women, aged between 15 and 60 years, attending gynecological clinics in our city from February 2005 to February 2006. The obtained results were correlated to the existent cytopathological and clinical data.

Gynecological and colposcopic examinations were performed upon routine consultation, with the techniques established in clinical practice. Papanicolaou smear examination was performed in a cytology laboratory upon medical requisition. HPV detection was performed in the same way in samples of cervical cells collected by the physician in a clinical laboratory using the consensus primers MY09 and MY11, and genotyping cervical cells collected by the physician in a clinical laboratory HPV detection was performed in the same way in samples of cervical cells collected by the physician in a clinical laboratory.

Discussion and Conclusions

In our study the prevalence of HPV in women undergoing routine cervical cancer screening was 30%, lower than that found by other authors [5, 17]. This difference is probably due to the social and economical level of the studied population, originating almost entirely from private clinics, which in our country poses a remarkable difference, and also because of the specific geographic characteristics of our region compared to the heterogeneous population of the country.

Among the women already presenting cytological alterations, most of the cases were classified as cervical...
intraepithelial alterations of low degree (7/68 CIN I), which is in agreement with the results found in other studies [18, 19]. A remarkable finding was the occurrence of seven patients with negative a HPV result, but presenting moderate- to high-degree alterations at the cytopathological examination (5/68 NIC II, 1/68 Class II and 1/68 NIC III). After a review of these cases with the respective physicians, we could evaluate that, invariably, those were cases with cytological/colposcopic alterations treated before the specimen collection for HPV detection, with the aim of monitoring therapeutics.

Most of the cases with no alterations in the Papanicolau test presented a negative HPV result. However, an important number of patients without cytological alterations presented HPV infection, being of low-risk (6/57, \( p = 0.0084 \)) and high-risk genotypes (2/57, \( p = 0.0804 \)), with one case with an indeterminate genotype. This indeterminate genotype, which was high-risk, would lead statistical analyses to a significant level of evidence (\( p = 0.0436 \)) of infection in women with no alterations in colposcopic or cytological examinations in our population. These data highlight the importance of early HPV detection in the prevention of cervical cancer, once development of neoplasia is definitively associated with the presence of these viruses.

Another contribution of HPV detection and typing in cervical samples of women undergoing gynecological assistance is the identification of an eventual previous infection by the virus, and in monitoring the efficacy of HPV vaccination. As is known, the commercially available vaccine so far protects only against four genotypes: 6, 11, 16 and 18 [20]. Our results are in accordance with other previous studies which demonstrate an epidemiological HPV profile only reasonably defined, being observed among the high-risk genotypes, a higher prevalence of HPV 16, followed by HPV 52, 51, 31, and others, with scarce findings of HPV 18 [21]. However, some epidemiological differences are noticeable in relation to the distribution of HPV genotypes in different geographic locations around the globe [22, 23]. HPV 18 seems to be more prevalent only in certain populations [24]. This epidemiological profile typical of each population may have important implications in vaccination efficacy. A women eventually infected by an HPV genotype not included in the vaccine should be constantly monitored for the development of cervical intraepithelial neoplasia, such as in the cases of infection with the high-risk HPV genotypes 31, 33, 45 and 52 found in our study. It is also important to remember that even HPV genotypes considered of intermediary risk may be associated with high degree intraepithelial alterations [25].

In brief, these data indicate that HPV detection and typing may constitute a useful early approach in prevention of uterine cancer, once an important number of women without colposcopic or cytological alterations may be infected by HPV, such as the 15.8% found in our study, including high-risk genotypes (3.5%). These cases must be monitored much more carefully in comparison to women without colposcopic or cytological alterations and a negative HPV result.

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References


Figure 1. — Prevalence of HPV genotypes identified by PCR-RFLP, in cervical samples of women undergoing uterine cancer screening. (N.I.: indeterminate RFLP).


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Maternal serum mannose-binding lectin in severe preeclampsia

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Summary
Purpose: We aimed to investigate the level of serum mannose-binding lectin (MBL) in severe preeclamptic patients, women with uncomplicated pregnancies, and healthy reproductive-age females and its impact on gestational age at delivery and birth weight.

Methods: Serum MBL levels were measured in 27 severe preeclampsia patients (Group 1), 27 patients with uncomplicated pregnancies (Group 2), and 25 healthy reproductive-age women (Group 3). Results: The mean serum MBL was significantly higher (p ≤ 0.05) in Group 1 than in Groups 2 and 3, while the levels in Groups 2 and 3 did not significantly differ. The mean gestational age at delivery and mean birth weight were significantly lower in Group 1. In Group 1, serum MBL was negatively correlated (p ≤ 0.05) with the gestational age at delivery and birth weight. Conclusion: Serum MBL increased in preeclampsia and was negatively correlated with the gestational age at birth and birth weight, indicating an underlying immunopathogenesis.

Key words: Mannose-binding lectin, Preeclampsia.

Introduction
In approximately 5% of cases, labor is complicated by preeclampsia – a disease that is unique to pregnancy and associated with maternal and fetal morbidity and mortality [1]. Preeclampsia is commonly manifested as hypertension and proteinuria in the second half of pregnancy [2]. Although its pathophysiology remains obscure, it is considered an inappropriate maternal immunological response against the fetal allograft [3, 4]. Traces of excessive maternal inflammation that cause defective trophoblast invasion and placentation can be seen both in the tissue and circulation [5-8]. Mannose-binding lectin (MBL) is one of the factors involved in the innate immune system [9]. MBL plays an important role against microorganisms by binding their carbohydrate moieties and activating the complement system via the lectin pathway [10, 11]. It is also a strong modulator of the complement-independent pathway and apoptosis [12].

Only a few studies evaluating the serum level of MBL and its genetic expression in some pregnancy-related complications have been reported in the literature [13-16]. Considering the proposed immunopathogenesis of preeclampsia and the importance of MBL in innate immunity, we investigated serum MBL levels in severe preeclamptic patients and compared them to the levels in patients with uncomplicated pregnancies and healthy reproductive-age females.

Materials and Methods
Twenty seven severe preeclampsia patients (Group 1), 27 pregnant women without any complications (Group 2), and 25 healthy reproductive-age women (Group 3) were included in the study. All the pregnant women had completed more than 28 weeks of gestation. Women with type 1, type 2, or gestational diabetes mellitus, rheumatological diseases, chronic renal disease, local or systemic infections, premature labor, premature rupture of membranes, and multiple gestations were excluded from the study.

Participating women provided informed consent, and the study was deemed to be in accordance with Helsinki declaration II and approved by the ethical committee of Uludag University Medical Faculty.

Severe preeclampsia was defined as blood pressure greater than 160/110 mmHg, as measured on at least two occasions six hours apart and proteinuria greater than 2 (+), as assessed using a dipstick (> 300 mg/dl) on two occasions. We centrifuged 5 ml venous blood from each patient at 3000 rpm, and the separated serum for MBL determination was stored at −20°C until the end of the study. Serum MBL was measured using enzyme-linked immunosorbent assay (ELISA) (KIT 030, Antibodyshop, Grusbakken 8, DK-2820 Gentofte, Denmark). ELISA was performed in microwells coated with mannose from Saccharomyces cerevisiae. The serum specimens were diluted in a calcium-containing buffer. Aliquots of calibrators and diluted serum samples were incubated in mannose-precoated microwells at room temperature for one hour. Functionally active MBL present in the solutions bound to the mannose-coated wells via its carbohydrate-binding domains. Unbound material was removed by washing. Biotinylated monoclonal detection antibodies were added to each test well and incubated at room temperature for one hour. The detection antibody attached to the bound MBL oligomers via the carbohydrate-binding domains that were not occupied during binding to the mannose coat. Unbound detection antibody was removed by washing. Horseradish peroxidase (HRP)-conjugated streptavidin was added to each test well and allowed to form a

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complex with the bound biotinylated antibody at room temperature for one hour. The unbound conjugate was removed by washing. A chromogenic peroxidase substrate containing tetramethylbenzidine (TMB) was added to each test well and incubated in a dark environment for 15 minutes. The bound HRP-streptavidin reacted with the substrate to generate a colored product. The enzymatic reaction was stopped chemically, and the color intensity was read at 450 nm in an ELISA reader. The color intensity (absorbance) indicated the concentration of the functionally active MBL originally added to each well. The results for the calibrators were used to construct a calibration curve from which the concentrations of functionally active MBL in the test specimens were read.

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) v13.0. The distribution patterns of the groups were analyzed using the Shapiro-Wilk test. The t-test was used in the comparison of two groups; and one-way analysis of variance, in comparison of more than two groups with normal distribution. The Mann-Whitney U test was used in the comparison of two groups, and the Kruskal-Wallis test in the comparison of more than two groups without normal distribution. The categorized data were analyzed using Pearson chi-square test and Fisher’s exact test. Pearson’s correlation coefficient was calculated, and correlation analysis was performed to determine any association between the variables. Statistical significance was defined at \( p < 0.05 \).

**Results**

As seen in Table 1, the demographic characteristics of the groups were comparable (\( p > 0.05 \)) with regard to the mean ages and the number of abortions. The mean gravidia was significantly (\( p < 0.001 \)) lower in Group 3 than in Groups 1 and 2, while those in Groups 1 and 2 did not significantly differ (\( p > 0.05 \)). As expected, the mean systolic and diastolic blood pressures in Group 1 were significantly (\( p < 0.001 \)) higher than those in Groups 2 and 3, while the blood pressures in Groups 2 and 3 did not significantly differ (\( p > 0.05 \)). The mean (± SD) Esbach value in the severe preeclampsia group was 3.1 ± 0.2 g/day. The mean serum MBL level in Group 1 was significantly higher (\( p < 0.05 \)) than those in Groups 2 and 3, while the difference between the levels in the latter two groups was not significant (\( p > 0.05 \)).

As seen in Table 2, the mean gestational ages at the time of serum sampling in Groups 1 and 2 were comparable (\( p > 0.05 \)); in contrast, the mean gestational age at delivery and the mean birth weight were significantly (\( p \leq 0.001 \)) lower in Group 1. The rate of cesarean section was significantly higher in Group 1 as opposed to that in Group 2 (\( p < 0.05 \)), but the ratios of the gender of the newborns in these groups were comparable (\( p > 0.05 \)) (Table 2). Acute fetal distress was the most frequent (78%) indication for cesarean section in the severe-preeclampsia group.

Serum MBL level was negatively correlated with the gestational age at delivery (\( r = -0.57, p \leq 0.01 \)) and the birth weight (\( r = -0.52, p \leq 0.01 \)) in Group 1. No correlation with age, gravidia, proteinuria, systolic and diastolic blood pressures was seen in the groups (\( p > 0.05 \)).

**Discussion**

Pregnancy is a unique situation in which a fetus with paternal alloantigens can survive in a uterus that is equipped with the maternal immune system. Trophoblasts invade the maternal spiral arteries and construct the feto-maternal interface by extending chorionic villi into sinuses formed by remodeled spiral arteries. The villi coated by syncytiotrophoblasts float freely within the maternal circulation for exchange transport of nutrients [1, 5]. This intrepid presentation of paternal antigens to the maternal immune system activates the complement system, and complement components physiologically accumulate in the placenta [17, 18]. This immune process is restrained by the development of immune tolerance, but the deposits of complement-activation products increase in a redundant manner in pregnancies complicated by preeclampsia [19, 20].

MBL is an important modulator of the innate immune system, and it participates in the activation of the complement system via the lectin pathway, in the modulation of inflammation, promotion of apoptosis, and removal of immune complexes and apoptotic cells [10, 21, 22]. MBL was detected on the endothelium of the spiral arteries [23]. Kilpatrick et al. [24] reported that MBL deficiency might be associated with recurrent miscarriages (RM). They found low levels of MBL in 16% of female partners and 14% of male partners among couples with RM compared to < 5% of controls. In 1999, Christiansen et al. confirmed the findings of this study [15]. They reported a highly significant (\( p < 0.01 \)) correlation between the magnitude of exposure (frequency of MBL deficiency) and the severity of the disease (number of miscarriages).
and proposed that MBL deficiency in women and RM might be causally related. However, although the frequency of MBL deficiency was higher in both Danish and Scottish women with RM than in controls, the difference was only significant when the two groups were combined. In 1999, Kilpatrick et al. tested 218 females and 179 male partners among couples with RM and 376 blood donors as controls [16]. In this study, they suggested that only MBL values ≤ 0.1 mg/ml should be considered clinically significant risk factors for spontaneous abortion. In 2002, Kruse et al. detected a significantly increased prevalence of low MBL levels in women with RM, irrespective of the cutoff level for low MBL, whether 50 or 100 ng/ml [25]. They hypothesized that abnormalities in the classic complement pathway might result in impaired immune-complex elimination and that since MBL activates the complement pathway, its deficiency might predispose to RM. In our study, we did not aim to elucidate the association between serum MBL levels and abortion rates. However, the mean numbers of abortions in the groups were comparable, and no correlation was seen between the serum MBL levels and the number of miscarriages (p > 0.05).

In 2006, van de Geijn et al., found that during an uncomplicated pregnancy, the serum MBL concentration increased to 140% compared to the baseline, which was defined as the concentration at six months postpartum [26]. This increase was present in the first trimester and did not significantly increase as the pregnancy advanced; on the contrary, it declined at six weeks postpartum. However, our findings were not in agreement with those of Geijn et al. Although the mean serum MBL level in women with uncomplicated pregnancies tended to be higher than that in healthy reproductive-age women, this difference did not reach statistical significance (p > 0.05). The study by Van de Geijn et al. was unique; however, a major bias in their study could be that the same patients served as the controls after delivery [26]. Further, they did not measure the patients’ pregestational serum MBL levels. Instead, they compared the gestational levels with the postpartum levels assuming that the patients would completely recover by the 6th postpartum month and that these values could be accepted as the nongestational baseline values.

Few studies reported in the literature have investigated the MBL status in preeclampsia. MBL can be studied directly by measuring the actual serum level or indirectly by determining the relevant genotypes. Sziller et al. studied the MBL codon 54 gene polymorphism, which is compatible with low MBL production, and concluded that carriage of this polymorphism protected against preeclampsia [13]. They hypothesized that an MBL-mediated event might be involved in the pathogenesis of preeclampsia. In contrast to the findings of Sziller et al., Van de Geijn et al. observed no association between the MBL genotypes and preeclampsia [14]. In our study, we measured the serum levels of MBL, but did not investigate the genotypes because they show varying expressions. In contrast to Sziller and Van de Geijn, we detected significantly higher mean serum MBL levels in the severe preeclamptic patients compared to the other two groups, maybe as a reflection of increased oxidative stress and inflammation (p < 0.05). Because there is not so much literature about serum MBL levels in uncomplicated gestation and preeclampsia, we measured serum levels in all three groups of patients. We found some increase in serum MBL levels during pregnancy but that did not reach significance when compared to the healthy reproductive-age group; hence the significant increase in the severe preeclampsia group was attributed to the toxemia itself.

Deficient maternal MBL concentration has also been described as a risk factor for preterm birth and reduced birth weight [27]. Kruse et al. reported that the median birth weight tended to be lower among women with MBL levels of 100 ng/ml than among women with normal MBL levels; however, the difference was not significant [25]. Furthermore, when they excluded the preterm births, they observed that the birth weights of the term infants born to women with MBL levels ≤ 100 ng/ml were significantly lower as compared to those of infants born to women with normal MBL levels. On the contrary, in a recently published report, van de Gieijn et al., investigated whether MBL polymorphisms play a role in preterm birth [28]. Serum MBL levels are in close association with single nucleotide polymorphisms in the structural gene. Individuals with the AA wildtype genotype have the highest MBL serum concentrations, and individuals with the OO genotype, which represent the variant alleles B, C and D, the lowest [29]. Van de Gieijn et al., have found that the high MBL production genotype group A was associated with shorter gestational age at delivery (274 days) compared to the intermediate MBL production genotype group B (283 days) and the low MBL production group genotype C (284 days). High MBL genotype group A constituted 86% of the preterm births, however there was no significant difference in gestational ages among the groups when the analysis was restricted to the women that gave birth at term. In our study, the mean gestational age at delivery and the mean birth weight were significantly (p ≤ 0.001) lower in the severe-preeclampsia group compared to that in the uncomplicated-pregnancy group. The finding that severe preeclampsia is associated with increased rate of preterm deliveries was not surprising [30]. The important finding, in our study, was the presence of negative correlations between the serum MBL level and gestational age at delivery (r = −0.57, p ≤ 0.01) and birth weight (r = −0.52, p ≤ 0.01) in the severe-preeclampsia group, which may indicate an underlying immune pathology.

Some authors have reported that decreased MBL levels are harmful to pregnant women, while others have reported contradictory findings; perhaps, the truth lies somewhere inbetween [14, 25]. High levels of MBL in normal pregnancy may indicate a physiological role acquired during evolution. Placentation is a complex process involving trophoblast invasion and spiral artery remodeling. It is a physiologic wound, and healing and
remodeling are prolonged, probably lasting throughout gestation. During this process, new tissue formation and remodeling occur repeatedly. MBL may play a dual role during the entire process. Its deficiency may decrease inflammatory reaction by decreasing the rate of complement activation, which is necessary for trophoblast invasion and attachment of the fetus to the uterus, and may result in miscarriage. Defects in the removal of inflammatory debris may contribute to the immunopathogenesis. However, increased MBL levels beyond physiological limits may increase the activity of the complement system that can destroy the maternal-fetal interface and induce preeclampsia.

In conclusion, we found that the serum MBL level was increased in preeclampsia, and that it negatively correlated with the gestational age at birth and the birth weight, indicating an underlying immunopathogenesis. However, it should be remembered that the serum MBL level is influenced by any inflammatory event such as trauma and infection. Because the knowledge regarding the association between MBL and preeclampsia reported in the literature is incomplete, further studies involving larger groups of patients are required.

References


Association of Fas-670 gene polymorphism with risk of cervical cancer in North Indian population

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Summary

Objectives: Cervical cancer is the second most common cancer among women in the world, with approximately 470,000 new cases and 231,000 deaths occurring each year. Incidence is greater in developing countries such as India, where this is the most common female malignancy with almost 100,000 new cases each year. Apoptosis must be considered as a safe mechanism that controls the integrity of the cell erasing abnormal clones and it is likely that failure of apoptosis constitutes a key factor responsible for tumor formation, progression and resistance to drugs. The Fas gene plays a key role in regulation of apoptotic cell death and corruption of this signaling pathway has been shown to participate in immune escape and tumorgenesis. Study design: A single-nucleotide polymorphism at -670 of Fas gene promoter (A/G) was examined in a total of 400 blood samples from normal healthy women and cervical cancer patients, using polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) technique. Results: Significant association was observed for AG (OR = 3.0, 95% CI = (1.68-5.09, p < 0.001) and combined AG+GG (OR = 2.54, 95% CI = 1.47-4.40, p < 0.001) genotype with risk of cervical cancer. Heterozygous genotype (AG) in SCC showed a highly significant association with risk of cervical cancer (OR = 2.57, 95% CI = 1.47-4.50 p < 0.001). Similarly, combined AG+GG genotype had a 2.25-fold risk for SCC patients (OR = 2.25, 95% CI = 1.30-3.90, p < 0.001). There was high increase risk of cervical cancer in passive smokers with AG and combined (AG+GG) genotypes (OR = 4.6, 95% CI = 2.07-10.32, p < 0.001 - OR = 4.9, 95% CI = 2.20-10.32, p < 0.001), respectively. Conclusion: This is the first study to provide evidence for the association of a Fas -670 (A/G) gene polymorphism with the risk of cervical cancer in a North Indian population.

Key words: Fas gene; Polymorphism; Cervical cancer.

Introduction

Cervical cancer is the second most common cancer in women worldwide, and is a preventable and a curable disease - especially if identified at an early stage. It is widely accepted that specific human papillomavirus (HPV) types are central etiologic agents of cervical carcinogenesis. Other environmental and host factors also play decisive roles in the persistence of HPV infection and further malignant conversion of cervical epithelium [1]. Although many previous reports have focused on HPV and environmental factors, the role of host susceptibility to cervical carcinogenesis is largely unknown. Smoking exposes the body to many carcinogens that affect more parts of the body than the lungs. Smoking contributes to a weakening of the immune system and tobacco by products have been found in cervical mucosa in women who smoke [2]. One recent study has found that women who smoke and have oncogenic HPV with abnormal Pap tests were more likely to be diagnosed later with precancerous or severe cervical dysplasia (CIN III) or cancer compared to nonsmokers [3]. Researchers believe that these substances damage the DNA of cervical cells and may contribute to the development of cervical cancer. Smokers are about twice as likely as non-smokers to get cervical cancer; however, the exact biologic relationship of smoking to oncogenic HPV is less clear [4]. Exposure to passive cigarette smoking is potentially modifiable, and hence this may have implications for strategies to prevent cervical cancer. The results of several case-control and cross-sectional studies have indicated that women married to smokers experience a higher risk of cervical neoplasia than those married to nonsmokers [5]. Apoptosis is a physiological process that regulates normal homeostasis, and alterations of apoptosis-related genes are likely to contribute to the pathogenesis of autoimmune disease [6] and malignant tumors [7]. Among various cell surface death receptors, Fas (CD95/APO-1) has a key role in known apoptosis pathways and is a member of the tumour necrosis factor receptor superfamily. The binding of Fas-L to the cell surface death receptor Fas activates intracellular cascades that ultimately cause apoptotic death of the cell [8, 9]. Downregulation of Fas with resultant resistance to death signals has been reported in many cancers [10]. The transcriptional expression of Fas gene is regulated by a number of genetic elements located in the 5' upstream region of the gene. The promoter region of the Fas gene consists of basal promoter, enhancer, and silencer regions [11]. Single nucleotide polymorphism at -670 in the enhancer region (A/G) situated at a binding element of gamma interferon activation signal (GAS), G allele results in an abolishment of the GAS element and a significant decrease in Fas gene expression in response to interferon (IFN-gamma) stimuli. The A allele has been
associated with autoimmune diseases [12, 13] and cervical carcinogenesis [14]. The Fas -670 A allele has also shown a higher binding affinity for the signal transducer and activator of transcription (STAT) 1 protein [15]. This would then lead to a reduction of CTL response which is beneficial for HPV in establishing persistent infections.

The aim of the study was to further investigate the Fas receptor SNP as a susceptibility factor for cervical carcinoma in a North Indian population.

Materials and Methods

Study subjects

This case-control study involved collection of peripheral blood samples (2-5 ml) from 400 North Indian subjects. The 200 cases were newly diagnosed, previously untreated and histologically confirmed as cervical cancer patients. The samples were collected from the Post graduate Institute of Medical Education and Research (PGIMER), Chandigarh and the Government Medical College (GMC), Chandigarh. The control peripheral blood samples (n = 200) were collected from the same institute with no history of cancer or precancer.

Informed consent was obtained from all the cases and controls. Detailed data regarding age, menarche, and menopausal status, number of children, age at marriage and birth of first child, cigarette smoking history and spouse’s smoking history were also obtained.

Methods

Genomic DNA was extracted from EDTA anti-coagulated peripheral blood samples according to a standard proteinase K digestion and phenol chloroform extraction method [16]. The Fas-G670A polymorphism was typed as described previously by Huang et al. [17] with the following minor modifications in polymerase chain reaction: 5 min at 95°C, 30 cycles of 30 sec at 95°C, 30 sec at 62°C, and 1 min at 72°C, followed by a final extension for 7 min at 72°C. Primer sequences were 5'-CTA CCT AAG AGC TAT CTA CCG TTC-3' and 5'-GGC TGT CCA TGT TGT GGC TGC-3'. The 332 bp PCR product was digested with MvaI for 5 hrs at 37°C. Allele G yielded three fragments of 99 bp, 189 bp, and 44 bp, whereas allele A yielded two fragments of 99 bp and 233 bp. Digested fragments were separated on 3% agarose gels and visualised after ethidium bromide staining.

Age, age at marriage and at birth of first child, cigarette smoking history and spouse’s smoking history were also obtained.

Results

The genotypes of the Fas gene at the -670 in cervical cases and healthy controls derived from a North Indian population was analysed.

Demographic variables for such cases and controls have been summarised in Table 1. The variables have also been categorised for squamous cell carcinoma (SCC) and adenocarcinoma (AC) cervical cancer; 175 were identified as SCC and 25 as AC.

The average age in years was calculated to be 48.55 ± 9.43 and 48.81 ± 9.64 for cases and controls, respectively. Compared with the controls, the study group was younger at the time of the marriage (16.36 ± 3.03) and birth of the first child (18.39 ± 3.39) and had a greater mean number of children (4.11). Ages at menarche and menopause were found to be comparable between cases and controls.

There were no significant differences in the Fas-670 genotype distribution and allelic frequencies between the healthy Indian controls and the other four healthy ethnic control groups (Table 2).

As shown in Table 3, the incidence of Fas-670 wild genotype (AA) in cases were lower (13.0%) than controls (27.5%). The distribution of heterozygous genotype (AG) was higher among cases (83.5%) than controls (60.5%). The association between the Fas-670 gene and cervix cancer has been summarised in Table 3. A significant association was observed for AG genotype (OR = 3, 95% CI = 1.68-5.09, p < 0.001) and risk of cervical cancer. Also a statistically significant risk (OR = 2.54, 95% CI =

Table 1. — Demographic characteristics of cervix cancer cases and controls.  
<table>
<thead>
<tr>
<th>Variable</th>
<th>Cases (200)</th>
<th>Controls (200)</th>
<th>SCC (175)</th>
<th>AC (25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age ±</td>
<td>48.55 ± 9.43</td>
<td>48.81 ± 9.64</td>
<td>48.39 ± 9.42</td>
<td>49.68 ± 9.64</td>
</tr>
<tr>
<td>Age at menarche ±</td>
<td>14.87 ± 1.14</td>
<td>14.02 ± 1.09</td>
<td>14.90 ± 1.16</td>
<td>14.68 ± 1.00</td>
</tr>
<tr>
<td>Age at marriage ±</td>
<td>16.36 ± 3.03</td>
<td>20.31 ± 3.46</td>
<td>16.68 ± 2.97</td>
<td>17.47 ± 2.56</td>
</tr>
<tr>
<td>Age at first</td>
<td>18.39 ± 3.39</td>
<td>22.31 ± 4.30</td>
<td>18.61 ± 3.36</td>
<td>16.84 ± 4.45</td>
</tr>
<tr>
<td>Age at birth ±</td>
<td>4.11</td>
<td>2.50</td>
<td>4.09 ± 1.51</td>
<td>4.21 ± 1.84</td>
</tr>
<tr>
<td>Age at smoking status</td>
<td>48.31 ± 5.56</td>
<td>48.26 ± 2.39</td>
<td>48.29 ± 3.62</td>
<td>48.44 ± 3.40</td>
</tr>
<tr>
<td>- non smoker</td>
<td>110 (55.0)</td>
<td>138 (69.0)</td>
<td>99 (56.6)</td>
<td>11 (44.0)</td>
</tr>
<tr>
<td>- active smoker</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>- active + passive smoker</td>
<td>2 (1.0)</td>
<td>6 (3.0)</td>
<td>2 (1.1)</td>
<td>0</td>
</tr>
<tr>
<td>- passive smoker</td>
<td>88 (44.0)</td>
<td>56 (28.0)</td>
<td>74 (42.3)</td>
<td>14 (56.0)</td>
</tr>
<tr>
<td>OR</td>
<td>2</td>
<td>1.0</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.002</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AC, adenocarcinoma; CI, confidence interval; OR, odds ratio; SCC, squamous cell carcinoma. Significance set at p < 0.05.

Table 2. — Fas-670 genotype and allele frequencies in several healthy ethnic controls.  
<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>AA</th>
<th>AG</th>
<th>GG</th>
<th>A</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chinese</td>
<td>41 (33)</td>
<td>64 (52)</td>
<td>19 (15)</td>
<td>59</td>
<td>41</td>
</tr>
<tr>
<td>Dutch</td>
<td>46 (23)</td>
<td>118 (57)</td>
<td>42 (20)</td>
<td>51</td>
<td>49</td>
</tr>
<tr>
<td>Australian</td>
<td>46 (25)</td>
<td>97 (53)</td>
<td>40 (22)</td>
<td>52</td>
<td>48</td>
</tr>
<tr>
<td>Korean</td>
<td>25 (30)</td>
<td>46 (55)</td>
<td>13 (15)</td>
<td>57</td>
<td>43</td>
</tr>
<tr>
<td>Indian</td>
<td>55 (27.5)</td>
<td>121 (60.5)</td>
<td>24 (12.0)</td>
<td>58</td>
<td>42</td>
</tr>
</tbody>
</table>

AA, adenocarcinoma; AG, adenocarcinoma and squamous cell carcinoma; GG, squamous cell carcinoma; A, adenocarcinoma; G, squamous cell carcinoma. Significance set at p < 0.05.
Table 3. — Fas-670 genotypes in cervical cancer and healthy controls.

<table>
<thead>
<tr>
<th>FAS genotypes</th>
<th>Case (%)</th>
<th>Control (%)</th>
<th>OR (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>26 (13.0)</td>
<td>55 (27.5)</td>
<td>1.0 (ref)</td>
<td>–</td>
</tr>
<tr>
<td>AG</td>
<td>167 (83.5)</td>
<td>121 (60.5)</td>
<td>3.0 (1.68-5.09)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GG</td>
<td>7 (3.5)</td>
<td>24 (12.0)</td>
<td>0.62 (0.21-1.76)</td>
<td></td>
</tr>
<tr>
<td>AG+GG</td>
<td>174 (87)</td>
<td>145 (72.5)</td>
<td>2.54 (1.74-4.04)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 4. — Determinants of interaction between Fas-670 genotypes and type of cervical cancer.

<table>
<thead>
<tr>
<th>FAS-670 genotypes</th>
<th>Type of cancer</th>
<th>OR (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>intact</td>
<td>1.0 (ref)</td>
<td>–</td>
</tr>
<tr>
<td>AG</td>
<td>SCC</td>
<td>2.57 (1.47-4.50)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GG</td>
<td>SCC</td>
<td>0.62 (0.21-1.76)</td>
<td>0.003</td>
</tr>
<tr>
<td>AG+GG</td>
<td>SCC</td>
<td>2.25 (1.30-3.90)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>AC</td>
<td>0.29 (0.14-0.56)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Table 5. — Assessments of interaction between Fas-670 genotypes and smoking in cervical cancer cases and controls.

<table>
<thead>
<tr>
<th>FAS genotypes</th>
<th>Status of smoking</th>
<th>Case %</th>
<th>Control %</th>
<th>OR (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>Never + Active +</td>
<td>14 (12.7)</td>
<td>34 (24.8)</td>
<td>1.0 (ref)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Passive</td>
<td>12 (46.2)</td>
<td>18 (32.7)</td>
<td>1.62 (0.56-4.72)</td>
<td></td>
</tr>
<tr>
<td>AG</td>
<td>Passive active</td>
<td>70 (79.5)</td>
<td>37 (66.1)</td>
<td>4.6 (2.07-10.32)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GG</td>
<td>Passive</td>
<td>6 (6.8)</td>
<td>1 (1.8)</td>
<td>3.0 (1.72-5.02)</td>
<td>0.007</td>
</tr>
<tr>
<td>AG+GG</td>
<td>Passive</td>
<td>76 (86.4)</td>
<td>38 (67.8)</td>
<td>4.9 (2.20-10.32)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Discussion

In the present study, we genotyped Fas-670 polymorphism in the North Indian population with cervical cancer and healthy controls, and found that the polymorphism is associated with cervical cancer. This study suggests that Fas-670 polymorphism might play a role in susceptibility of cervical cancer in the North Indian population. There is an expanding body of literature suggesting that host factors, including genetic polymorphisms, may explain some of the individual differences in cancer occurrence. Polymorphisms in the promoter region or 5' flanking region of genes can lead to different levels of gene expression and have also been implicated in a number of diseases. SNP at -670 of the Fas gene promoter (A/G) has been found with potentially different transcriptional efficiency [18]. Since this polymorphism of the Fas gene is located in the promoter region, it may affect the level of transcription of the Fas protein. Bauvois et al. [19] suggested that the substitution of G to A in the position -670 (TTCCAG G/AAA) would change the gamma interferon activation site (GAS) (TTC-nnnGAA). This site was involved in interferon gamma and interferon alpha signaling [20]. GAS elements are known to bind to homodimers of a phosphorylated form of the 91-kDa transcription factor, STAT1. Interferon gamma could cause tyrosine phosphorylation of STAT1 by the interferon gamma receptor-associated Janus kinases 1 and 2. Subsequently, phosphorylated STAT1 formed homodimers and translocated into the nucleus where it induced transcription of GAS containing genes [21]. Fas has been significantly upregulated by interferon gamma according to several reports [22]. Several studies have addressed the association of this SNP with autoimmune disease and Fas promoter -670 polymorphism analysis on cervical cancer showed that the frequency of A allele and AA genotype increased in accordance with the multiple step carcinogenesis [23]. Ueda et al. [24] suggested that Fas gene promoter -670 polymorphism (A/G) may be closely associated with cervical carcinogenesis in a Japanese population. Also, they reported that there was an increased OR for AG+GG genotype in HSIL cases compared to controls among the patients with high-risk HPV. The same trend was observed in our study in that AG+GG genotype increased the risk of developing cervical cancer (OR 2.54, 95% CI = 1.47-4.40, p < 0.001) in North Indian women. Engelmark et al. [25] and Dybikowska et al. [26] have demonstrated that AA genotype in the Fas gene promoter at -670 position may not be engaged in the development of cervical neoplasia in Swedish and Polish populations. These discrepancies may be due to the ethnic variation of HPV prevalence and genotype frequency of the Fas gene promoter in different geographical regions. Zhang et al. [27] demonstrated that gene-environment interaction of the FAS polymorphism and smoking was associated with increased risk of lung cancer. Similarly, results raised in this study in passive smoking cancer patients with AG and GG genotypes of Fas -670, (OR - 4.6, CI, 95% = 2.07-10.32, p < 0.001, OR = 3.0, CI, 95% = 1.72-5.02, p = 0.007), respectively. The -670 Fas polymorphism has been reported to be associated with Alzheimer’s disease and to interact with the apolipoprotein – E variant [28], indicating that it has potential biological significance. However Fas poly-
phism does not appear to have an impact on non-melanoma skin cancer [29]. Also Xia et al. [30] could not find any significant association between Fas-670 polymorphism and inflammatory bowel disease in Chinese patients. It is imperative to test if the polymorphism could be used as a disease marker for the natural history of cervical lesions in the setting of a longitudinal cohort study. Functional analysis of Fas-670 polymorphism in infiltrating lymphocytes and stromal cells from patients with precancerous lesions will be important in order to understand molecular mechanisms precipitating to cervical carcinogenesis. The limitation of the present study is that it was hospital-based and took place in that environment, therefore it can not be free from any selection bias. In conclusion, to the best of our knowledge, this is the first study to date that provides evidence for an association between Fas gene polymorphism and risk of cervical caner in a North Indian population.

Acknowledgments

The authors are grateful to all staff and the patients who took part in this study. We are also thankful to PGIMER and GMCH Chandigarh for providing the clinical samples.

References


Silicone gel mammary prostheses: immune pathologies and breastfeeding

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¹Department of Surgical Sciences, ²Department of Gynecology and Obstetrics, University of L’Aquila, Faculty of Medicine and Surgery, L’Aquila (Italy)

Summary

Augmentation mammoplasty is the most frequent request among esthetic surgery procedures but numerous controversies have been raised about the security of the silicone gel prostheses. Today a new question needs an answer: is the prosthesis a risk factor for pregnancy? In this paper the results of a hematocchemical study performed on a group of patients with term pregnancies and silicone gel breast implants (group A) compared with a control group without implants (B) are described. For laboratory screening the valuation of antibody (TRIM) and silicone concentrations in blood and maternal milk and in neonate blood was performed.

Key words: Augmentation mammoplasty; Mammary prostheses; Autoantibody; Silicone; Immune pathologies; Breastfeeding.

Introduction

Augmentation mammoplasty has been the object of numerous controversies: could mammary prostheses be considered as a risk factor for women during pregnancy, lactation or fetal development? Could silicone during the puerperium contaminate the milk and predispose the newborn to immune pathology? [1-11]. The clinical trials present in the literature have attempted to answer these questions, but have often been affected by errors in the sampling. They often result without statistical significance or are contradictory [1-6].

Studies with small groups have little statistical significance whereas larger groups can determine immune pathology in an independent way from mammoplasty. The latter has statistical significance but often is contradictory and not reproducible.

The aim of our study was to attempt to answer these questions: the safety of silicone gel prostheses for the mother, the effects of eventual contamination of maternal milk and if this condition is associated with elevated concentrations of silicone in the blood of the neonate and, if silicone presence is correlated with immune pathology in the newborn.

Materials and Methods

The study was conducted jointly by the Department of Plastic and Reconstructive Surgery and the Department of Gynaecology and Obstetrics of L’Aquila School of Medicine (Italy). From January 1995 to December 2005, 15 women near term pregnancy with mammary silicone gel prostheses, (Group A) were selected. Exclusion criteria were fibrocystic mastopathy, mastitis, immune pathology determined before mammoplasty and all women with saline prostheses. Pregnancies secondary to assisted fecundation were excluded.

Table 1. — Clinical data.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Group A (n = 15)</th>
<th>Group B (n = 15)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>(with prostheses)</td>
<td>(without prostheses)</td>
<td> </td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>27.9 ± 4.13</td>
<td>26.8 ± 3.60</td>
<td>0.443</td>
</tr>
<tr>
<td>Gestation time</td>
<td>39.3 ± 4.12</td>
<td>39.4 ± 1.04</td>
<td>0.812</td>
</tr>
<tr>
<td>Duration of breastfeeding</td>
<td>26.1 ± 4.12</td>
<td>26.0 ± 4.71</td>
<td>0.937</td>
</tr>
<tr>
<td>Implant permanence</td>
<td>62.1 ± 32.3</td>
<td> </td>
<td> </td>
</tr>
</tbody>
</table>

A control group (B) included 15 women near term pregnancy without breast implants.

Group A had a mean age of 27.9 ± 4.1 years old. The mean time of implant permanence was 62.1 ± 32.3 months. Eleven patients (73.4%) had the prostheses in the subglandular plane and four (26.6%) in the submuscular plane. Mean pregnancy time was 39.3 ± 1.2 weeks and breastfeeding duration was 26.15 ± 4.1 weeks. Group B had a mean age of 26.8 ± 3.6 years old, a pregnancy rate of 39.4 ± 1 weeks and the mean duration of breastfeeding was 26 ± 4.7 weeks (Table 1).

At prepartum admission, evaluation of ESR, CRP, RF, Ig A,G and M classes and ANA/ENA antibody tests were performed in all patients.

The same laboratory parameters were carried out for the infants of our patients at the start and end of breastfeeding. We also evaluated silicone concentrations in the whole blood of the mothers and infants, and in the maternal milk.

The typical instruments were used to evaluate the concentration of antibodies, whereas silicone concentrations were evaluated by spectroscopic analysis with silicone-free devices [12, 13].

The results were statistically analyzed to verify the relation coefficient between the prosthetic inserts and the values of tests by the chi-square test, one-way analysis of variance and t-test.

Results

Values of inflammatory proteins are shown in Table 2. Antibodies in all groups were compared by the chi-square test and the difference was not significant (Table 3). Sil-
Table 2. — Inflammatory proteins.

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESR</td>
<td>5.9 ± 3.32</td>
<td>6.2 ± 3.39</td>
<td>0.788</td>
</tr>
<tr>
<td>C-RP</td>
<td>86.8 ± 19.91</td>
<td>85.4 ± 21.56</td>
<td>0.855</td>
</tr>
<tr>
<td>RF</td>
<td>0.142 ± 0.03</td>
<td>0.135 ± 0.03</td>
<td>0.588</td>
</tr>
</tbody>
</table>

ESR: erythrocyte sedimentation rate; C-RP: C-reactive protein; RF: rheumatoid factor.

Table 3. — Antibody profile.

|              | No. of patients |          |          |          |          |
|--------------|----------------|----------|----------|----------|
|              | ANA | ANA | ENA | ENA | (+) | (-) | (+) | (-) | (+) | (-) |
| Group A      | 3   | 12  | 2   | 13  | 7   | 8   | 7   | 8   | 5   | 10  |
| Group B      | 3   | 12  | 2   | 13  | 10  | 6   | 9   | 4   | 11  |
| $\chi^2$    | 0.208| 0.288| 0.139| 0.000| 0.000| 1.000| 1.000| 1.000| 1.000|
| p            | 0.648| 0.591| 0.709| 1.000| 1.000| 0.000| 0.000| 1.000| 1.000|

Table 4. — Silicone concentration.

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood</td>
<td>83.0 ± 41.50</td>
<td>80.9 ± 35.25</td>
<td>0.881</td>
</tr>
<tr>
<td>Maternal milk</td>
<td>51.1 ± 22.91</td>
<td>51.1 ± 18.60</td>
<td>0.998</td>
</tr>
</tbody>
</table>

Table 5. — One-way analysis of variance of silicone concentrations in the blood.

<table>
<thead>
<tr>
<th>Variation source</th>
<th>Degrees of freedom</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between groups</td>
<td>1</td>
<td>33.07</td>
</tr>
<tr>
<td>Within groups</td>
<td>28</td>
<td>1482.41</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.882</td>
<td></td>
</tr>
<tr>
<td>$F$</td>
<td>0.02</td>
<td></td>
</tr>
</tbody>
</table>

Table 6. — One-way analysis of variance of silicone concentrations in the milk.

<table>
<thead>
<tr>
<th>Variation source</th>
<th>Degrees of freedom</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between groups</td>
<td>1</td>
<td>0.00</td>
</tr>
<tr>
<td>Within groups</td>
<td>28</td>
<td>435.41</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>$F$</td>
<td>0.02</td>
<td></td>
</tr>
</tbody>
</table>

docrine concentrations in the whole maternal blood and milk are reported in Table 4; to compare and establish the significance level we performed one-way analysis of variance (Tables 5 and 6).

Silicone in maternal blood was more concentrated in patients in Group A, but the difference was not statistically significant. Silicone concentrations in the maternal milk of both groups were superimposable. Thus no correlation between mammary implants and silicone values in the milk were found.

Evaluation of the concentration of inflammatory proteins in newborn blood was performed both at the start and end of breastfeeding. The results were compared by the Student’s t-test (Tables 7 and 8).

Antibody rates were studied in the newborns of both groups in the same mode as the other tests and the statistical significance was evaluated by the chi-square test (Tables 9 and 10).

Table 7. — Comparison between concentration of inflammatory proteins in newborns at the start and end of breastfeeding.

<table>
<thead>
<tr>
<th></th>
<th>T test</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESR start</td>
<td>-0.386</td>
<td>0.702</td>
</tr>
<tr>
<td>ESR end</td>
<td>0.288</td>
<td>0.776</td>
</tr>
<tr>
<td>CRP start</td>
<td>0.000</td>
<td>1.000</td>
</tr>
<tr>
<td>CRP end</td>
<td>-0.078</td>
<td>0.938</td>
</tr>
<tr>
<td>RF start</td>
<td>-0.130</td>
<td>0.897</td>
</tr>
<tr>
<td>RF end</td>
<td>-0.091</td>
<td>0.928</td>
</tr>
</tbody>
</table>

ESR: erythrocyte sedimentation rate; C-RP: C-reactive protein; RF: rheumatoid factor.

Table 8. — Comparison between blood silicone concentrations in newborns at the start and end of breastfeeding.

<table>
<thead>
<tr>
<th>Hematic silicone concentration</th>
<th>T test</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start</td>
<td>0.417</td>
<td>0.680</td>
</tr>
<tr>
<td>End</td>
<td>1.321</td>
<td>0.197</td>
</tr>
</tbody>
</table>

Discussion

Silicon (Si) is one of the most common elements on the earth crust and traces can be found in food, make-up, drugs, clothes and also in the hair of some people [10, 14-18].

Numerous compounds in nature have a basis of Si but only the crystalline form has been able to define the pathogenesis because it is the cause of lung fibrosis and pleural mesothelioma [10, 14-18].

The organic form of Si, like silicone, is used to prepare prosthetic implants used in medicine [19].

Controversies on the safety of mammary implants made of silicone gel have been ongoing since 1980. Numerous case reports on patients with immune pathology were considered to be a consequence of augmentation mammoplasty. Consequently the FDA forbid the sale of silicone gel breast prostheses in February 1992 [1-6, 20].

In that period augmentation mammoplasty by silicone gel implants had been reserved only for patients undergoing mastectomy or volunteers enlisted in experimental trials [15-18].

This phenomenon brought about the start of numerous studies that affirmed or negated the relation between local and/or systemic illness and silicone gel prostheses.

Today exactly how silicone interacts with biologic tissues is not completely understood and how it acts as a trigger for immune pathology is even less understood [20, 21].

All prostheses, independent from the other substances added to silicone, induce a fibrous reaction in peri-prosthetic tissue thus indicating non tolerability to the silicone [15, 16, 22, 23].

Cases of lymph-node biopsies reported in the literature show that the presence of silicone depends on the phagocytosis process of silicone molecules by macrophages and the successive transport of the material to the lymphatics [20, 24-26].

Silicone captured by the macrophages could derive from premature prosthetic failure to the capsule formation or bleeding of the gel through the prosthesis envelope [16, 17, 27-29].
The aim of our study was to attempt to define the relation between silicone gel filled implants and the onset of immune pathology in carriers as well as their offspring.

Our results are in accordance with those found in the literature. Investigation of hematohemical and antibody markers, while helpful in the diagnosis of immune pathology, does not put in evidence any particular cause-effect relation and it is absolutely non specific for other clinical implications [4, 8, 31]. The same result was observed in the analysis of newborn antibody rates in Group A which did not have statistical significance in any test.

As for silicone concentrations in maternal whole blood we observed higher values in patients with prostheses. However this difference did not appear to be due to the silicone gel. Instead silicone concentrations in maternal milk and in the blood of newborns were the same in all groups suggesting a different mode of contamination.

Critical analyses of our cases are in agreement with the pathology, does not put in evidence any particular cause-effect relation: a cumulative experience of 11 cases". J. Rheumatol., 1993, 20, 958.


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Silicone gel mammary prostheses: immune pathologies and breastfeeding 189
Ultrasound assessment of endometrial thickness: correlation with ovarian stimulation and pregnancy rates in IVF cycles

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Assisted Reproduction Unit, 3rd Department of Obstetrics and Gynecology, University of Athens, “Attikon” Hospital (Greece)

Summary

Purpose: To study the correlation between endometrial thickness and IVF outcome and factors affecting this relation. Methods: Transvaginal ultrasound evaluation of endometrial thickness on hCG administration day in 112 IVF-ET cycles and comparison to indices of ovarian stimulation. Outcome was considered positive when fetal sac and fetal heart pulse were present at ultrasound. GnRH-agonist and antagonist protocols were also compared. Statistical analysis was performed by the SPSS system, chi-square and t-test. Results: 38 cycles displayed clinical pregnancy. In cases of higher endometrial thickness, pregnancy rates, mean serum estradiol levels, oocyte and mature oocyte numbers as well as mean large follicle numbers were higher, while the mean age was lower. Conclusions: In 38 cycles resulting in pregnancy, mean endometrial thickness was higher compared to cycles with negative outcomes. Higher serum estradiol is associated with higher endometrial thickness and pregnancy rates. Women achieving pregnancy and pregnant women with endometrium thicker than 9 mm were younger. Follicle stimulation was better with higher endometrial thickness. After adjustments for age, no statistical difference was found in endometrial thickness between agonist and antagonist protocols.

Key words: Endometrial thickness; Transvaginal ultrasound; IVF outcome; Ovarian stimulation.

Introduction

Endometrial receptivity and its relation to implantation of fertilized oocytes have been widely investigated by means of correlation with in vitro fertilization (IVF) outcome. Transvaginal ultrasound examination provides an easy and non-invasive way of assessing endometrial receptivity. Thickness, blood flow and pattern are related to endometrial receptivity and can be quite precisely determined by transvaginal ultrasound examination. Endometrial thickness is the most easily measured index.

Many studies have been conducted to compare endometrial thickness between successful and unsuccessful IVF cycles. Large studies (1,186 and 897 IVF cycles, respectively) have demonstrated that achieving pregnancy through IVF cycles is unlikely in cases of thin endometrium [1, 2]. Other investigators have not concluded to this relation, based either on small populations [3], or on stimulation protocols using clomiphene citrate combined with hMG (human menopausal gonadotrophin) [4].

The ideal endometrial thickness has also been investigated with most scientists suggesting that 7-14 mm is the best range [5], while others have not confirmed this limit, supporting that implantation and pregnancy rates do not significantly differ between endometrial thicknesses of more or less than 14 mm [6]. Factors affecting endometrial thickness have also been studied, such as elevated serum estradiol levels [7] and duration of ovarian stimulation [2].

The induction of ovarian stimulation in IVF, leading to the production of many follicles and as a result many oocytes, has increased IVF success rates [8]. Collection of many oocytes during oocyte retrieval is very much desirable. Speculation arises when follicles of different size and function are present, leading to oocytes of different maturation levels.

Ultrasound assessment of follicular size (diameter) can be used as an indicator of ovarian stimulation and oocyte maturity in IVF cycles. The size of large follicles after all is determinant for the time of hCG (human Chorionic Gonadotropin) administration, which promotes matura-

It is widely accepted though that ultrasound assessment of follicular size and serum estradiol levels in women undergoing IVF is the most reliable indicator of oocyte maturity in IVF cycles.

Furthermore, studies have been conducted to investigate and compare the effectiveness of GnRH-agonist and GnRH-antagonist protocols in IVF. The use of GnRH-antagonists in IVF cycles prevents a premature rise in serum LH levels in most women and rapidly inhibits secretion of gonadotropin and steroid hormones [12]. This conveys a potential advantage over GnRH-agonists in the management of ovarian stimulation. Researchers have compared pregnancy rates, ovarian stimulation and factors affecting the cycle outcomes between the two protocols.

The purpose of this study was to investigate the correlation between endometrial thickness, ovarian stimulation and pregnancy rates and to study the factors affecting this correlation, along with comparing GnRH-agonists to GnRH-antagonists.

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Materials and Methods

In the Assisted Reproduction Unit of Athens University, 3rd Department of Obstetrics and Gynecology at “Attikon” Hospital, 112 IVF cycles were studied out of those performed in our Unit during the year 2005. Using transvaginal ultrasound examination on the day of hCG administration we measured endometrial thickness in all cases and divided our measurements in two groups. Group A consisted of endometrial thickness less than 9 mm, while group B consisted of cases with endometrial thickness more than 9 mm. During the same ultrasound scan we assessed the number of follicles with a diameter > 17 mm.

Pituitary suppression was achieved by GnRH-agonists in 50 cases and GnRH-antagonists in 62 cases. Ovarian stimulation was achieved by recombinant FSH, hCG was administered when at least three follicles had reached the mean diameter of 17 mm and oocyte retrieval was performed after 36 hours. The numbers of oocytes, as well as mature oocytes (Metaphase II) were marked. Embryo-transfer was performed on day 2 or 3 of the cycle. Each cycle used each woman’s own oocytes.

To study the correlation between endometrial thickness and achieving pregnancy with IVF we considered the outcome positive when establishing by ultrasound the presence of a fetal sac and fetal heart pulse. Factors considered important in this study were stimulation duration, mean serum estradiol levels on the day of hCG administration, women’s age, total FSH dosage and protocol stimulation.

Statistical analysis was performed by the SPSS system. Quantitative control was based on the t-test, quality control was based on the chi-square method, and results were considered statistically significant when p < 0.05.

Results

Out of 112 cycles included in our study, clinical pregnancy rate per embryo transfer was determined in 34%, which corresponds to 38 cycles. The most important results derived, are quoted in Table 1.

Table 1. — IVF outcome.

<table>
<thead>
<tr>
<th>Pregnancy/ET</th>
<th>Follicles d &lt; 17 mm</th>
<th>Oocytes</th>
<th>Mature oocytes</th>
<th>Estradiol levels (pg/ml)</th>
<th>Age (years)</th>
<th>Stimulation duration (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mean ± SD)</td>
<td>(mean ± SD)</td>
<td>(mean ± SD)</td>
<td>(mean ± SD)</td>
<td>(mean ± SD)</td>
<td>(mean ± SD)</td>
</tr>
<tr>
<td>Group A</td>
<td>4 (5%)</td>
<td>2.1 ± 2.4</td>
<td>5.1 ± 3.1</td>
<td>4.6 ± 2.2</td>
<td>1074 ± 689</td>
<td>38.9 ± 3.9</td>
</tr>
<tr>
<td>E &lt; 9 mm</td>
<td>n = 20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group B</td>
<td>34 (37%)</td>
<td>4.1 ± 2.7</td>
<td>8 ± 3.2</td>
<td>8 ± 3.2</td>
<td>1813 ± 1040</td>
<td>31.7 ± 3.4</td>
</tr>
<tr>
<td>E ≥ 9 mm</td>
<td>n = 92</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
<td>NS</td>
</tr>
</tbody>
</table>


In group B, where endometrium was thicker than 9 mm, we found a statistically significantly higher pregnancy rate per embryo-transfer (37%) compared to the same rate in group A (5%) (p < 0.05). Women in group B also displayed higher (mean ± SD) serum estradiol levels (1813 ± 1040 vs 1074 ± 689 pg/ml, p < 0.05) and were younger than women in group A (mean ± SD) (31.7 ± 3.4 vs 38.9 ± 3.9 years old, p < 0.05). However a factor not found to be related to endometrial thickness was the duration of stimulation. The differences between the two groups were not statistically significant, with a slightly longer duration in group B (mean ± SD) (10.3 ± 2.4 vs 10 ± days).

Ovarian stimulation was more satisfactory in Group A. The mean number of follicles with a diameter higher than 17 mm, as well as the mean number of oocytes and mature oocytes were statistically significantly higher in cases of endometrial thickness > 9 mm.

Moreover, we ascertained that endometrial thickness was significantly higher in cases of positive outcome (fetal sac and fetal heart pulse in ultrasound examination), with a mean thickness of 11.2 mm compared to negative outcomes, where mean endometrial thickness was 9.8 mm (p < 0.05).

Comparing the effectiveness of GnRH-agonist and GnRH-antagonist protocols in these cycles we concluded that pregnancy rates did not show any statistically significant difference between the two protocols. The mean ± SD age was significantly higher in the antagonist group as compared to that of the agonists. By means of endometrial thickness though, GnRH-agonists showed a stronger association with thicker endometrium (Table 2), but after adjustment for differences of age this difference was not statistically significant.

Table 2. — GnRH-agonists vs GnRH-antagonists.

<table>
<thead>
<tr>
<th>Pregnancy/ET</th>
<th>E &gt; 9 mm</th>
<th>E &lt; 9 mm</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (%)</td>
<td>(mean ± SD)</td>
<td>(mean ± SD)</td>
<td>(mean ± SD)</td>
</tr>
<tr>
<td>GnRH-agonists</td>
<td>35%</td>
<td>(n = 52 (46%))</td>
<td>(n = 18)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(n = 50)</td>
<td>(n = 2)</td>
</tr>
<tr>
<td>GnRH-antagonists</td>
<td>30%</td>
<td>(n = 60 (54%))</td>
<td>(n = 20)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(n = 42)</td>
<td>(n = 18)</td>
</tr>
<tr>
<td>p</td>
<td>NS</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>


Discussion

According to our study results endometrial thickness is a factor very much related to the positive outcome of IVF cycles by means of achieving pregnancy. In 38 cycles resulting in pregnancy, mean endometrial thickness was statistically significantly higher compared to cycles with a negative outcome (11.2 mm vs 9.8 mm, respectively). Moreover, high serum estradiol levels have been found to be associated with higher endometrial thickness and consequently with higher pregnancy rates. Women having achieved pregnancy after IVF, as well as pregnant women whose endometrial thickness had been higher than 9 mm, were younger. Therefore, endometrial thickness is a factor determinant of the positive outcome in IVF cycles affected by serum estradiol levels, but according to our results not by the stimulation duration. We also investigated the potential link between the treatment protocol and endometrial thickness. We compared the agonist and antagonist protocols and after adjustment for differences in age, no statistical difference was found.

The role of endometrial receptivity in IVF-ET cycle outcomes has been widely investigated. Several studies are concordant to our findings, demonstrating that higher endometrial thickness is correlated to higher pregnancy rates. De Geyter et al. [1] suggested a fall in pregnancy rates when endometrium is thin, in a large series of 1,186
IVF cycles. In the study of Zhang et al. (897 IVF cycles) [2] the outcome was found superior in cases of higher endometrial thickness on the day of hCG administration. These investigators have also suggested that this seems to be affected by the duration of ovarian stimulation. The dependency of endometrial thickness on serum estradiol levels shown in our study has also been demonstrated by other investigators. They have all predicated that endometrial thickness is higher, when serum estradiol levels are higher.

Another trial has determined the particular endometrial thickness considered ideal for achieving pregnancy [5]. This retrospective study referred to 809 IVF-ET cycles in 623 women. The investigators divided their material in two groups based on endometrial thickness on the day of hCG administration. The first group consisted of cases with endometrial thickness of 7-14 mm and the second of cases with endometrial thickness higher than 14 mm. Successful implantation rates were found in 15% and 3%, respectively and pregnancy rates in 29.7% and 8.1%, respectively. Dietterich et al. [6] conducted a retrospective study of 570 women during which they evaluated endometrial thickness using transvaginal ultrasound on the day of hCG administration. Of those women 510 exhibited endometrial thickness lower than 14 mm, while the remaining 60 women displayed endometrial thickness higher than 14 mm. Successful implantation, pregnancy and spontaneous abortion rates were found to be similar between the two groups in that trial.

On the other hand some investigators have demonstrated that endometrial ultrasound parameters (thickness and pattern) do not differ between women achieving or not pregnancy after IVF cycles [13]. Some trials have not supported the correlation between endometrial thickness and IVF cycle success, referring though either to small populations [3] or to stimulation protocols using clomiphene and hMG (human menopausal gonadotrophin) [4].

Jarvela et al. [14] suggested that endometrial thickness is not a factor of great importance in predicting IVF outcome compared to endometrial pattern. The investigators established that the presence of triple-line endometrium after ovarian stimulation correlates to improved outcome. The same study demonstrated no differences between women achieving or not pregnancy by means of endometrial thickness, volume, and vascularization.

It has been endorsed that apart from thickness, endometrial receptivity is also designated by endometrial volume and vascularization. The role of endometrial volume and thickness in the prognosis of clinical pregnancy after assisted reproductive techniques (ART) has been explored in perspective. Higher endometrial volume (> 2 ml) and higher endometrial thickness have been shown to correlate to improved outcome. Investigators have suggested that endometrial volume constitutes a more significant factor in the prognosis of achieving pregnancy compared to endometrial thickness [15].

During recent years three-dimensional (3D) power Doppler ultrasound has been widely used to evaluate parameters determinant of endometrial receptivity apart from thickness, such as endometrial volume and endometrial-subendometrial vascularization. Studies conducted in order to display the role of endometrial-subendometrial vascularization in the prognosis of IVF outcome have demonstrated that vascularization is better in women achieving pregnancy by IVF, compared to negative IVF outcome [16].

Folicular size and endometrial thickness are definitely factors of great importance in the prognosis of IVF outcome and ovarian stimulation itself. Recently investigators have examined the role of folicular and ovarian blood flow ultrasound in the prognosis of IVF using Doppler. It seems that these parameters along with follicular size and endometrial thickness can be used for this purpose [17].

By means of achieving pregnancy our results suggest higher – but with no statistical significance – pregnancy rates for the cases of GnRH-antagonists. There has been wide investigation of this matter. Al Inany and Aboughar found a clinical pregnancy rate of 5% in favor of GnRH-agonists, which is concordant with our findings [18]. Meta-analyses on studies comparing the two protocols have yielded conflicting results as regards the likelihood of achieving pregnancy, the most recent of which suggested higher efficacy by using antagonists [19, 20]. Research cannot overlook though the fact that GnRH-antagonist cycles are more often conducted in older patients, which has important implications for the interpretation of findings.

New potentiality in evaluating prognostic factors for ART outcome can be provided by 3D and 4D ultrasound. The quest of prognostic parameters for IVF effectiveness is a field of wide and constant investigation. The prognostic value of factors affecting endometrial receptivity, such as volume and vascularization, are under exploration.

References


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Cranial imaging spectrum in hypertensive disease of pregnancy

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Summary

Objective: To determine cranial imaging findings in patients with severe preeclampsia, eclampsia and HELLP syndrome and the correlation between these findings and neurological symptoms. Materials and Methods: CT or MRI findings of 120 patients diagnosed with severe preeclampsia, eclampsia and/or HELLP syndrome between January 1998 and December 2005 are presented. Results: Pathological imaging findings were observed in 28.1% (n = 32) of the severe preeclampsia group, in 43.3% (n = 30) of the HELLP group, in 51.35% (n = 27) of the eclampsia group and in 61.9% (n = 21) of the eclampsia + HELLP group and in 45% of all patients. Thirty-five patients had specific pathology defined as ischemic lesions, edema, and perivascular microhemorrhage. Infarcts were found in seven, intracranial hemorrhage in seven, hydrocephaly in two, dural sinus thrombosis in two and a pineal cyst in one patient. Specific lesions were generally located in the posterior parietal and occipital lobes. Five patients died due to intracranial hemorrhage and one patient due to septic shock. Conclusion: A wide imaging spectrum from the ischemic lesion to severe intracranial hemorrhage can be detected in complicated cases of hypertensive diseases of pregnancy. It is essential to perform cranial imaging in patients with symptoms and neurological deficits.

Key words: Imaging spectrum; Pregnancy; Hypertensive disease.

Introduction

Severe preeclampsia, eclampsia and HELLP syndrome are complicated forms of pregnancy-induced hypertension with an incidence of 5-10% of pregnant women [1, 2]. Intracranial complications are the leading causes of maternal mortality and morbidity. Early diagnosis improves prognosis in patients with neurological symptoms. Clinical studies have indicated that intracranial pathologies are not rare, particularly in preeclamptic patients (3, 4). However, there were no studies comparing the cranial pathologies in severe preeclampsia, eclampsia and HELLP syndrome and correlations between cranial pathologies and symptoms have not been investigated before.

In the present study, we aimed to investigate cranial imaging findings of hypertensive disease during pregnancy and whether there were differences in spectrum and incidence of lesions.

Materials and Methods

Between January 1998 and December 2005, 120 patients who underwent computed tomography (CT) and/or magnetic resonance imaging (MRI) with a diagnosis of severe preeclampsia, eclampsia or HELLP syndrome at the Department of Obstetrics and Gynecology, Istanbul School of Medicine were included in the study. Symptoms of the patients were recorded and all patients were examined by a neurologist. Patients were grouped as Group 1 (n = 32) severe preeclampsia, group 2 (n = 30) HELLP syndrome, group 3 (n=37) eclampsia and group 4 (n = 21) eclampsia with HELLP syndrome.

All patients were examined carefully by a neurologist and cranial imaging was performed for clinically suspicious cases. MRI was performed in 48 patients and CT in 72 patients. Cranial imaging was performed within the first 24 hours in patients with eclampsia, and within the first 48 hours of diagnosis in other cases. Contrast medium was not used in any of the patients. The neuroradiologist who evaluated the radiograms was blinded to clinical details.

Results of the following laboratory tests were recorded at admittance: complete blood count, transaminase levels and coagulation parameters. Patients with intracranial hemorrhage were compared with the others regarding age, gestational age, symptoms, neurological findings, platelet count, transaminase levels and coagulation parameters.

The study was designed prospectively. Fisher’s exact chi-square, Student’s t and one-way ANOVA tests were used in statistical analyses. Level of statistical significance was defined as a p value of less than 0.05.

Results

Characteristics such as age, gestational age, parity, blood pressure values at admittance, clinical symptoms and pathological imaging findings for all groups are summarized in Table 1.

Imaging findings were evaluated as normal in 66 (55%) patients. Among the other 54 (45%) patients, the pathological findings were as follows: specific lesions which could be evaluated as edema, ischemic lesions or petechial hemorrhage in 35 (29.2%) cases; intracranial hemorrhage in seven (5.8%) cases; infarcts in seven (5.8%) cases; hydrocephaly in two (1.6%) cases; dural sinus thrombosis in two (1.6%) cases; and pineal cyst in one (0.8%) case. Distribution of findings according to the groups is shown in Table 2. The ratio of patients who had...
Pathological imaging findings was 28% in the severe preeclampsia group, 43.3% in the HELLP group, 51.5% in the eclampsia group and 61.9% in the eclampsia + HELLP group and there was a statistically significant difference between groups (p = 0.033).

Specific lesions were mostly in the posterior parietal and occipital lobes, temporal and frontal lobes and basal ganglia, in the cortex, white-gray matter intersection, subcortical white matter or in deep white matter. These lesions were seen as hypodense areas in CT, hypo or isointense foci in T1-weighted MRI sequences, but hyperintense foci in T2-weighted sequences.

Intracranial hemorrhage was observed in seven patients and five of these died. The patients who survived were operated on and discharged from the hospital with right hemiplegia and total aphasia. In all patients with intracranial hemorrhage the main symptom was sudden loss of consciousness (Table 3). When these patients were compared with those without intracranial hemorrhage, blood pressure at admittance, age, transaminase levels, symptom and neurologic finding ratio and FDP values were higher, and gestational age, platelet count and fibrinogen levels were lower (Table 4). Patients with intracranial hemorrhage had elevated PT and APTT values, although no significant difference between the groups was found (p > 0.005).

Clinical prognoses of patients were followed, but no imaging examination was needed except in two patients. One hundred and twelve (93%) patients were discharged as healthy without neurological symptoms; two patients with intracranial hemorrhage who underwent surgery were discharged with sequels. Five patients died of intracranial hemorrhage, and one patient died due to septic shock despite normal imaging findings. The general mortality rate was 5%.

**Discussion**

Neurological complications of hypertensive disease in pregnancy might be fatal [6]. With the use of high resolution CT and MRI, both major life-threatening pathologies and minor pathologies which do not alter clinical prognosis can be determined in early stages [2, 4]. Results of recent studies on the subject have differed. Sibai and colleagues [5] reported that the incidence of pathological imaging findings in 20 patients with atypical eclampsia was 0%, while Richard et al. [2] reported the incidence in 192 patients with eclampsia as 75%; Digre and colleagues [6] in 16 patients with severe preeclampsia as 50%. Our study was important since these four groups of patients were compared.

In the present study, no significant differences were found among severe preeclampsia, eclampsia, HELLP syndrome and eclampsia with HELLP syndrome groups regarding age, gestational age, parity and blood pressure at admittance (p > 0.05). On the other hand, increases in pathological imaging findings were significant (p < 0.05). If the diseases described above were evaluated as the stages of the same disorder, it may be concluded that in parallel to the severity of the disease, symptoms and cranial pathologies tended to increase.

Within the spectrum of pathological imaging findings
due to complications of hypertension during pregnancy, intracranial hemorrhage, diffuse cerebral edema, infarcts, hydrocephaly, dural sinus thrombosis and some other specific lesions have been observed [6, 9-11]. The disturbance patterns of lesions observed in the present study were in accordance with the literature, but no diffuse cerebral edema was seen in any of our cases. In addition, pathologies such as pineal cyst (in one patient) and hemorrhagic infarct (in one patient) not related to pregnancy were observed.

Specific lesions affecting mostly the middle and posterior cerebral artery perfusion areas were thought to represent hypoxic brain damage, perivascular hemorrhage, perivascular microinfarcts, and particularly edema [12]. Specific lesions are generally reversible and do not affect the patient’s prognosis [9, 10]. Occipital and parietal lesions were mostly bilateral and symmetrical, whereas temporal lobe, frontal lobe and basal ganglia lesions were infrequently bilateral and generally asymmetrical. The reason for the predominant involvement of the posterior circulation areas is not clear, but it has been reported that the vessels which supply posterior circulation have less sympathetic innervations than those of anterior circulation and therefore the permeability of these vessels increases due to the loss of autoregulation during hypertensive attacks [13-16].

Intracranial hemorrhage is the most severe complication in hypertensive complications of pregnancy and it usually causes sequels in surviving patients [11, 17]. In the present study, seven (5.8%) patients had intracranial hemorrhage; five of these died and the other two patients lived with hemiplegy and total aphasia. In these patients, several common features were observed. The diagnosis of these seven patients was not only preeclampsia but it was also complicated by eclampsia or HELLP syndrome [HELLP syndrome (n = 2), HELLP + eclampsia (n = 3), eclampsia (n = 2)]. Hemorrhage localization of the patients who did not survive was in the brainstem or it flowed to the 3rd, 4th and arterial ventricles. In one patient hepatic intraparenchymal hematoma was determined.

Loss of consciousness: LOC.

Table 3. — Characteristics of patients with intracranial hemorrhage.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Age (yrs)</th>
<th>Gestational age (years)</th>
<th>Complaint</th>
<th>Blood pressure (mmHg)</th>
<th>Hemorrhage localization</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eclampsia with HELLP syndrome</td>
<td>37</td>
<td>24</td>
<td>LOC</td>
<td>210/160</td>
<td>In white matter at right parieto-occipital area (8 x 7 x 4 cm) and in brainstem (2 x 3 cm)</td>
<td>Died on 5th day</td>
</tr>
<tr>
<td>Eclampsia with HELLP syndrome+ oligo-hydramnios HELLP syndrome</td>
<td>27</td>
<td>38</td>
<td>LOC</td>
<td>180/100</td>
<td>In left parietal lobe (7 x 3 cm)</td>
<td>Operated, right hemiplegic and totally aphasic</td>
</tr>
<tr>
<td>Eclampsia with type II diabetes HELLP syndrome</td>
<td>34</td>
<td>28</td>
<td>LOC</td>
<td>190/120</td>
<td>In left parietal lobe (4 x 5 cm) and also in the liver</td>
<td>Died on 5th day</td>
</tr>
<tr>
<td>Eclampsia with HELLP syndrome</td>
<td>39</td>
<td>28</td>
<td>LOC</td>
<td>280/160</td>
<td>In left putamen (5x5 cm) which flowed to the 3rd ventricle Originated from the brainstem and flowed to the 3rd, 4th and arterial ventricles</td>
<td>Died on 4th day</td>
</tr>
<tr>
<td>Eclampsia with HELLP syndrome</td>
<td>30</td>
<td>26</td>
<td>LOC</td>
<td>180/100</td>
<td>Brain stem and in the left parieto-occipital area</td>
<td>Died on 4th day</td>
</tr>
<tr>
<td>HELLP syndrome</td>
<td>32</td>
<td>24</td>
<td>LOC</td>
<td>220/130</td>
<td>Left parietal lobe</td>
<td>Operated, discharged with right hemiplegy</td>
</tr>
</tbody>
</table>

Table 4. — Comparing of patients with and without intracranial hemorrhage regarding age, gestational age, blood pressure, symptoms, neurological findings and laboratory values.

<table>
<thead>
<tr>
<th></th>
<th>Patients with intracranial hemorrhage (n = 7)</th>
<th>Patients without intracranial hemorrhage (n = 113)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>33.5 ± 4.85</td>
<td>27.28 ± 5.62</td>
<td>0.014</td>
</tr>
<tr>
<td>Gestational age</td>
<td>28.7 ± 4.80</td>
<td>33.41 ± 4.71</td>
<td>0.032</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>209.27 ± 43.18</td>
<td>160.41 ± 19.15</td>
<td>0.0001</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>129.32 ± 28.45</td>
<td>105.12 ± 13.65</td>
<td>0.0003</td>
</tr>
<tr>
<td>Symptom (%)</td>
<td>100 (7/7)</td>
<td>56 (64/113)</td>
<td>0.0240</td>
</tr>
<tr>
<td>Neurological finding (%)</td>
<td>100 (7/7)</td>
<td>39 (45/113)</td>
<td>0.0170</td>
</tr>
<tr>
<td>Platelets</td>
<td>67.200 ± 34.80</td>
<td>180.540 ± 55.730</td>
<td>0.0002</td>
</tr>
<tr>
<td>SGOT (U/l)</td>
<td>145.63 ± 70.42</td>
<td>46.65 ± 17.42</td>
<td>0.0001</td>
</tr>
<tr>
<td>SGPT (U/l)</td>
<td>135.26 ± 45.62</td>
<td>46.43 ± 11.98</td>
<td>0.0001</td>
</tr>
<tr>
<td>PT</td>
<td>16.25 ± 4.86</td>
<td>12.9 ± 5.01</td>
<td>0.223</td>
</tr>
<tr>
<td>APTT</td>
<td>36.32 ± 6.15</td>
<td>30.12 ± 6.50</td>
<td>0.0723</td>
</tr>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>270.95</td>
<td>375.66 ± 70.31</td>
<td>0.0017</td>
</tr>
<tr>
<td>FDP (μ/ml)</td>
<td>45.57 ± 8.65</td>
<td>31.70 ± 8.30</td>
<td>0.0018</td>
</tr>
</tbody>
</table>
consciousness developed suddenly in all patients with intracranial hemorrhage, and death occurred between four and six days. When compared with the patients without intracranial hemorrhage, these patients were significantly older and had higher blood pressure at admission, and lower gestational age. As a consequence, we thought that high blood pressure, older maternal age and younger gestational age increased the severity of preeclampsia and therefore, risk for intracranial hemorrhage.

It was reported in the literature that MRI was superior to CT to determine intracranial pathologies due better contrast and resolution in the images [6, 18, 19]. Although CT was the first method to diagnose acute hemorrhage, it was stated that MRI was more sensitive to demonstrate chronic and subacute small residual hemorrhage.

We believe that cranial imaging should be performed in selected patients with neurological findings and undetermined clinical status. No imaging examinations are needed in asymptomatic patients or patients without any neurological findings, since such cranial lesions are temporary and reversible, and there is no specific therapy for these lesions. Patient symptoms should be questioned and neurological examination is required for symptomatic patients. Patients with severe symptoms such as loss of consciousness, confusion, loss of motor power or focal neurological findings should be examined immediately with CT to mainly diagnose any hemorrhage. MRI should be reserved for patients with a stable clinical situation or for patients with persistent symptoms despite normal CT findings.

Acknowledgement

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References

The effect of buserelin acetate on the uterus of adult rats: morphological aspects

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Summary

Purpose of investigation: To evaluate the effect of buserelin acetate on the morphology of the endometrium of adult, non-castrated, female Wistar rats. Methods: Female Wistar rats at estrus or diestrus (assessed by vaginal cytology) received daily subcutaneous injections of 20 mg buserelin acetate for four, eight or 12 days. Rats were sacrificed 24 hours or five days following final dosage. A control group received diluent for 12 days. Results: Progressive tissue hypotrophy occurred during treatment and was followed by estrogenic hyperactivity five days after the end of treatment. Vaginal cytology and endometrial histology revealed intense, vacuolized lining and glandular epithelia, brush borders and endometrial stroma densely infiltrated with eosinophils. Conclusions: Buserelin acetate appears to cause a progressive blockade of gonadotrophin secretion when administered to female rats for four, eight or 12 days, and an important rebound effect, with accentuated estrogen release already apparent in the first estrous cycle following treatment.

Key words: Buserelin; Gonadotrophin; Female rats; Estrous cycle; Cytology; Endometrium; Eosinophilia.

Introduction

Over the last 30 years, further research on GnRH analogs has confirmed the molecular stability of these substances, as well as their greater affinity for pituitary receptors, their longer half-life, enzymatic resistance and increase in ovulatory potential. Different effects can be achieved by varying their dosage, route of administration, duration of use and the posology of the drug. When administered intermittently, they induce the pulsatile release of the follicle stimulating hormone (FSH) and luteinizing hormone (LH). When used continuously, there is a strong initial stimulus followed by subsequent gonadotrophin desensitization by receptor endocytosis, resulting in reversible hypogonadotropic hypogonadism [1, 2].

This capacity of gonadotrophins to display either a stimulatory or an inhibitory effect has permitted the ample clinical use of GnRH analogs (GnRH-a), particularly in in vitro fertilization [3], prostate cancer [4], breast cancer [5], uterine myomas, endometriosis [6] and precocious puberty [7].

With respect to side-effects, GnRH-a may cause hot flashes, flushing, weight gain, nausea, vaginal atrophy, discomfort, myalgia, cephalgia, vomiting, depression, mood swings, acne, premenstrual syndrome, decreased libido, abdominal pain, mastalgia, asthenia, irritability, dizziness, dry skin, constipation, dyspepsia, gynecomastia and coughing [6].

In view of the important gonadotrophin stimulating and inhibiting capacity of GnRH analogs, the objective of this study was to evaluate the onset of these effects by assessing the morphological changes to the uterus and vagina induced by a GnRH-a. Female Wistar rats that reach sexual maturity at a mean of 92 days, have a gestation of 22 days and an estrous cycle of 3.7-5 days’ duration, similar to that of humans with respect to variations in LH, FSH and prolactin [8], were used in the study. The estrous cycle of the rat is characterized by being regular, periodic and coordinated, and the histology of its reproductive tract is well-defined. The phases of diestrus, proestrus, estrus and metestrus are characteristic, with vaginal cytology, the basic structure of the uterine wall, vascular architecture and leukocyte and eosinophil infiltrates undergoing predictable periodic variations. The endometrium is the area in which the greatest physiological variations in reproductive activity occur (Table 1) [8-10].

Materials & Methods

Female adult Wistar rats (Rattus norvegicus albinus), aged between 82 and 102 days, and weighing 250-330 grams were used in this study. They were maintained in confinement, isolated from males following gender identification, and fed with unrestricted chow and drinking water. The rats were introduced into the study during diestrus and estrus (Figure 1), identified using wet mount vaginal cytology. These phases were chosen because they coincide with, respectively, the lowest and highest synthesis and release of endogenous gonadotrophins. The study protocol was approved by the local ethics committee for the control of research studies involving laboratory animals in accordance with the IRB of the School of Medicine, Federal University of Minas Gerais.

For cytology, three drops of 30% toluidine blue were deposited in the vagina of the rats using an automatic pipette tip attached to a rubber dropper, and material was immediately col-
The effect of buserelin acetate on the uterus of adult rats: morphological aspects

lected for examination using the thick drop method between a slide and a coverslip under an optical microscope. This procedure was repeated every three days until completion of the required number of rats in each study group, and then again on the day the animals were sacrificed. The definition of the estrous phase is based on the proportions between nucleated cells, keratinized cells and leukocytes obtained from the vaginal cavity (Table 1).

The GnRH analog used was Suprefact®, supplied by Hoechst do Brasil Quimica e Farmacêutica S.A., São Paulo, SP, Brazil, for subcutaneous use, composed of 1 mg of buserelin acetate and 10 mg of benzyl alcohol diluted into 1 ml of vehicle. Each animal received 20 μg of buserelin acetate subcutaneously at a volume of 0.1 ml following dilution of the drug. The control group received 0.1 ml of the vehicle commercialized in Suprefact®, also supplied by Hoechst and labeled “placebo”. The estrous cycle of four days and the dose of buserelin acetate used were based on observations in the literature from studies carried out by Long & Evans and Lobel et al. [9, 10]. Using a 1 ml disposable syringe and a 4x13 needle, the rear paw of the rat was transfixed for the injection in the subcutaneous region opposite the point of puncture, avoiding reflux and loss of the product. Table 2 describes the flowchart for vaginal cytology, administration of buserelin acetate and sacrifice of the rats in experiment A, beginning during diestrus, and experiment B, beginning during estrus.

The rats were sacrificed following inhalation of anesthesia with sulfuric ether. Samples were collected for vaginal cytology, after which the abdominal cavity of the rat was opened to remove the uterus, ovaries, bladder and the upper third of the vagina. Under macroscopy using a pachymeter, the length of the smallest uterine horn and the length of its mid-third were measured. Next, the uterus was placed on filter paper on a Petri dish and fixed in 10% formaldehyde. Three fragments were obtained from the mid-third of the smallest uterine horn, and these were embedded in paraffin and stained with hematoxylin-eosin and picrosirius, the latter for the detection of collagen in the tissue.

Using an optical microscope, the muscle layers of the uterus and the endometrium were examined, paying particular attention to the glandular and lining epithelia and to the stromal cellularity. Morphometry was used to measure the height of the lining and glandular epithelia, and the thickness of the endometrial stroma and the muscle layer, as well as the internal area of the uterus. All measurements were taken at the 12, 3, 6 and 9 o’clock positions using an ocular micrometer calibrated with a 1 mm rule divided into 100 subdivisions of 10 μg each. The measurement used was the arithmetical mean of the measurements taken at the four established points of reference. For the intensity of dilatation and vascular congestion of the uterus, the stromal cellularity, presence of intraepithelial leukocytes, eosinophils in the stroma and the proportion of nucleated cells, keratinized anucleated cells and leukocytes in the vaginal cytology, a score was established as follows: slight (+), moderate (++), and strong (+++).

Statistical analysis was performed using non-parametric tests for the comparison of different groups with each other, using the statistical software program GB-Stat Professional Statistics & Graphics, version 4.0 (Dynamic Microsystems, Inc., Silver Spring, USA). Differences were considered significant when p < 0.05.
pseudo-stratified, and strong infiltration of eosinophils in the endometrium, with highly vacuolized brush borders, leukocyte and eosinophilic infiltrate, high columnar epithelium, intense edema in the vessels and glands, strong absence of leukocytes. There was strong uterine dilatation (anucleated cells), few nucleated cells and hyperactivity. Vaginal cytology showed intense cell keratination.

On the contrary, in the rats sacrificed at the end of the first estrous cycle following 12 days of treatment, irrespective of whether the experiment was initiated in the diestrus or in the estrus, the results of cytology and uterine morphology were characteristic of estrogenic hyperactivity. Vaginal cytology showed intense cell keratinization (anucleated cells), few nucleated cells and absence of leukocytes. There was strong uterine dilatation, intense edema in the vessels and glands, strong leukocyte and eosinophilic infiltrate, high columnar endometrium, with highly vacuolized brush borders, pseudo-stratified, and strong infiltration of eosinophils

In the groups treated with buserelin acetate for a period of approximately one to three estrous cycles (4-12 days) and sacrificed 24 hours following administration of the final dose, cytology revealed a great number of leukocytes, nucleated cells and scarce or no keratinized anucleated cells. There was a progressive reduction in estrogenic activity. In the groups sacrificed after one estrous cycle following the end of treatment, cytology was characterized by an abundance of keratinized, anucleated cells that corresponded to practically all the cells in the smear. This aspect of cytology is compatible with strong estrogenic activity. It emphasizes not only the immediate return of gonadotrophic secretory activity following the use of buserelin acetate but also an intense gonadotrophic response, typical of a rebound effect.

In the first four days of treatment, the results of the two experiments initiated during diestrus and estrus were similar, with a slight estrogenic stimulus and a small increase in the height of the glandular epithelia and epithelial lining and in the thickness of the muscle layer. In the animals treated for more than four days, the uterus tended towards diestrus, i.e., with no dilatation in the cavity, no vascular congestion, and a cuboid endometrial and glandular epithelium, with scarce cell exudate in the stroma. This confirms that buserelin acetate effectively induces a progressive blockade of gonadotrophin production, giving the uteri the morphological characteristics of organs that have not undergone hormonal stimulation. This progressive blockade was very marked in the animals treated for 12 days, where values of epithelial height were lower, indexes of eosinophilic exudation were lower in the endometrium and vaginal cytology was typical of diestrus.

The animals sacrificed after one estrous cycle without medication had morphological data indicative of intense estrogenic uterine activity. The lining and glandular epithelium were high, vacuolized, with brush borders,
and the endometrial stroma was intensely infiltrated by eosinophils. The presence of eosinophils is a morphological finding strongly indicative of estrogenic hyperactivity in the rat [10]. Dilatation and the accumulation of liquid are also morphological findings typical of uteri under the influence of estrogen. These observations permit us to conclude that the inhibition of gonadotrophic secretion induced by buserelin acetate may, when the drug is withdrawn, go through a typical rebound phenomenon.

Conclusion

The experiments show that buserelin acetate injected subcutaneously in the female rat at a dose of 20 μg/day for more than one estrous cycle tends to result in vaginal cytology and uterine morphology compatible with a state of low gonadotrophic stimulation, resembling diestrus.

In contrast, four days after cessation of the treatment, there seems to be a release of gonadotrophins apparently greater than that of a normal cycle, a fact confirmed by the pronounced alterations in vaginal cytology and in uterine morphology that are compatible with estrogenic hyperactivity.

In the experiment initiated during diestrus, the weight gain curve followed the same pattern as during estrus, but percentages of weight gain were clearly greater in the first group. On the other hand, the rats in the untreated groups in both experiments underwent no variations with respect to percentage of weight gain, confirming that buserelin acetate may result in a greater weight gain in rats when administration is initiated during diestrus. We were unable to find any satisfactory explanation for this finding in the literature.

Acknowledgements

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References


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Use of GnRH antagonists in ovarian remnant syndrome experimentally induced in rats

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Summary

Purpose: The objective of this study was to demonstrate the efficacy of cetrorelix, a GnRH antagonist, in rats with experimentally induced ovarian remnant syndrome.

Methods: 25 Wistar female rats at seven to eight weeks of age and weighing 200-250 g were used. The rats were randomly divided into five groups: the first group was used as a control group; the second and third groups underwent a sham operation; and the fourth and fifth groups underwent bilateral hemiovariectomy. At the first proestrus detected by vaginal cytology from postoperative day 2, the animals in groups 1, 2 and 5 received placebo and the animals in groups 3 and 4 received cetrorelix subcutaneously. In the study, the Kruskal-Wallis analysis of variance was used for comparison of the results of vaginal irrigation, histopathological examination, and of blood FSH and LH values, and the Mann Whitney U-test was used for determination of the differences between the groups.

Results: It was determined that according to vaginal cytology results, estrus-like cytological changes disappeared in a shorter time and according to histopathology results, the number of follicles were fewer in the ovarian remnant syndrome-induced and cetrorelix-injected group 4 (p < 0.05), but there was no difference between the groups for FSH and LH concentrations.

Conclusions: Ovarian remnant syndrome is a complication of bilateral ovariohysterectomy. In cases with this syndrome, certain treatment is possible with re-operation. However, it may not always be possible to perform an operation, or even if operated, it is difficult to determine the place of the residual ovarian tissue. In this study, it was determined that the use of cetrorelix as a GnRH antagonist in rats with ovarian remnant syndrome reduced the duration of estrogenic affect.

Key words: GnRH antagonist; Ovarian remnant syndrome; Rat.

Introduction

GnRH analogues are derived by deletion of the primary structure of GnRH or by shifting the location of one or more amino acids. These analogues include GnRH agonists and antagonists. GnRH antagonists bind to GnRH receptors in the hypophysis with a great affinity; however, they cannot cross-bind to the GnRH receptor and thus cannot induce calcium-mediated gonadotropin release. Consequently, they depress the LH secretion in a short time, strongly and reversibly [1-4].

Up to date, three different types of GnRH antagonists have been developed. The first two are not much used as they cause local and systemic reactions by inducing histamine release. Third generation GnRH antagonists induce a lower release of histamine while strongly depressing ovulation. Cetrorelix and ganirelix are the most frequently used third generation antagonists. The effects of GnRH antagonists on LH release and ovulation have shown variation according to dose and sexual stage in studies on both women and rats [4-8].

Ovarian remnant syndrome is a complication of ovariectomy and ovariohysterectomy, which occurs when functional tissue of the ovary remains after the operation. Breed, race, age, physical condition of the female, difficulty of the operation, or the experience of the surgeon are not related with the occurrence of this syndrome. The most important symptom of ovarian remnant syndrome is the continuous estrus activity in animals and menstrual activity in women following ovariectomy and ovariohysterectomy. This syndrome is important in females because follicular activity can show an increase in the residual ovarian tissue, consequently resulting in follicular cyst development, which will lead to an increase in the estrogen concentration that will cause pelvic pain, vaginal bleeding and more importantly, aplastic anemia in bitches [9-11].

The objective of this study was to determine whether GnRH antagonists can be used as a temporary treatment alternative in the termination of symptoms related to ovarian remnant syndrome.

Materials and Methods

Animals

In this study, 25 Wistar female rats of at seven to eight weeks of age and weighing 200-250 g were used. The animals were obtained from the Experimental Research Center of the Faculty of Medicine of Firat University. Throughout the study, the animals were housed in cages, each containing five rats; a 12-hour dark and 12-hour light regime was followed. The animals were given feed and water ad libitum. Animals with regular sexual cycles, confirmed by four consequent vaginal irrigations, were used for the experiments.
Use of GnRH antagonists in ovarian remnant syndrome experimentally induced in rats

Operations

Rompun (10 mg/kg IM) – ketalar (90 mg/kg IM) anesthesia was induced during the operations and the animals were randomly divided into five groups.

The animals in the first group (n = 5) were selected as the control group and did not undergo any operation.

Animals in group 2 (n = 5) and group 3 (n = 5) underwent the sham operation. Animals in group 4 (n = 5) and group 5 (n = 5) underwent bilateral hemiovariectomy

Treatment protocol

At the first proestrus detected by vaginal cytology carried out as of postoperative day 2, the animals in groups 1, 2 and 5 received placebo, and the animals in groups 3 and 4 received cetrorelix (Cetrotide, Serono, Istanbul Turkey) subcutaneously. The dose of cetrorelix was 0.46 mg/kg/single administration [12].

Vaginal irrigations

The sexual cycles of all animals were followed by Giemsa-stained preparations prepared from the vaginal irrigations taken in 4-hour intervals following the injections until the estrus signs disappeared cytologically. The intensities of cell types in the prepared samples were assessed as +, ++, +++ [13].

Histopathological Examination

The residual ovarian tissues of rats sacrificed by decapitation at the cytological start of diestrus were sent to the laboratory in 10% buffer formaldehyde. Following the routine tissue procedures, tissue sections with 4 μg thickness were prepared and stained with hematoxylin-eosin for histopathology examinations. The preparations were examined for the presence of follicles under magnification (40 x) of an Olympus Bx50 light microscope. The primordial follicles were confirmed by the presence of at least one-fold of cubical follicle cells, while the antral follicles were confirmed by the presence of an antrum within the follicle [14].

Laboratory analysis

Blood samples were obtained from rats at the time when diestrus began cytologically and the sera were analyzed by ELISA for FSH and LH concentrations [15].

Statistical analysis

In the study, the Kruskal-Wallis analysis of variance was used for comparison of the results of vaginal irrigation, histopathological examination, and blood FSH and LH values, and the Mann Whitney U-test was used for determination of the differences between the groups [16]. These statistical analyses were carried out using the SPSS statistical package (Release 9.0, 1999).

Results

Results obtained from the study are presented in Table 1, Figure 1 and Figure 2. According to vaginal cytology and histopathology results, a difference was found among the groups (p < 0.01, Kruskal-Wallis analysis of variance), and cytological estrus signs disappeared earliest in group 4 (6.40 ± 1.60 hours) (p < 0.05, Mann Whitney U-test). In addition, group 4 had the minimum follicle number according to histopathology results (6.00 ± 1.31 piece) (p < 0.05, Mann Whitney U-test). However, no difference was found among the groups for FSH and LH concentrations (p > 0.05, Kruskal-Wallis analysis of variance).

Discussion

A certain alternative treatment for ovarian remnant syndrome is possible by extirpation of residual ovarian tissue by reoperation. However, difficulty in the determination of residual ovarian tissue is the primary problem in such operations. In addition, in cases where the operation is a
contraindication due to general condition disorders arising from generalized infections, alternative treatments can be applied with medicines such as hCG, megesterol acetate and mibolerone [17-19].

Rivier and Vale [20] have reported that in rats with a one-week pregnancy, GnRH antagonists led to a decrease in LH concentrations, concurrently leading to a decrease in progesterone concentration at a degree that caused abortions following the application in the 7th-12th days of pregnancy. In the present study, the LH concentration in the hemiovariectomized cetrorelix group (group 4) showed a 42% reduction compared to the placebo group. However this reduction was not statistically significant. It has been suggested that in ovarian remnant syndrome, the use of cetrorelix leads to a decreasing tendency of LH concentration.

Although not yet used in ovarian remnant syndrome, the antagonistic effect of ganirelix has been investigated in many studies in both males and females. It has been reported that its administration at a 1.4 ug/kg dose to female rats at the proestrus stage depresses ovulation. Furthermore, it has been suggested that ganirelix administration at doses as high as 0.7-5.0 mg/kg/day depress estrus completely and irreversibly [21]. In the present study, cetrorelix was used for the first time in rats with experimentally-induced ovarian remnant syndrome. According to the vaginal cytology and histology results, it was determined that estrogenic signs in the cetrorelix rat group (group 4) disappeared in a shorter time than in the other groups.

GnRH antagonists can rapidly inhibit the release of FSH and LH in all stages of the sexual cycle. This situation will assist in termination of the negative effects of endogenous estrus by blocking the follicular development in cases with ovarian remnant syndrome where surgery is a contradiction. In addition, termination of antagonist use will lead to an increase in hypophysal activity, which will consequently promote follicular development in the ovaries in cases with ovarian remnant syndrome, which will make the determination of residual ovarian tissue easy during the operation. In conclusion, in the present study, it has been determined that the use of cetrorelix as a GnRH antagonist in rats with ovarian remnant syndrome reduced the duration of the estrogenic effect, reduced the number of follicles in the ovary, and led to a decreasing trend of LH concentrations. In light of the obtained results, more advanced studies are needed on the efficacy and benefits of GnRH antagonists in cases with ovarian remnant syndrome.

References
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Usefulness of symphysis-fundal height in predicting fetal weight in healthy term pregnant women

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²Surgical Division of Obstetrics and Gynecology, San Giovanni Battista Hospital-ASL 3 Foligno (Italy)

Summary

Background: There are some works reporting that the measurement of the symphysis-fundal height (SFH) of a full-term uterus is a simple method for estimating the fetal weight. Aim: Evaluating the goodness of distance between the symphysis and uterine fundus in predicting both low-weight fetuses and high-weight fetuses, comparing it with the third quarter ultrasound estimation of fetal weight and then assessing the clinical effectivity of symphysis-fundal measurement associated with third quarter echography in predicting birth weight. Methods: A prospective study was carried out on 96 single physiologically full-term pregnancies. The diagnostic accuracy of the SFH, echographic fetal growth estimated between the 32nd and the 35th week (expressed in percentiles), and of both was expressed as sensitivity, specificity, predictive positive and negative value, likelihood ratios and compared. Results: There was a correlation between the SFH and fetal birth weight. A SFH below 33 cm is predictive of a fetus whose weight is less than 3,100 g whereas a SFH above 34 cm is predictive of a fetus whose weight is more than or equal to 4,000 g. The diagnostic effectiveness of the SFH was not significantly higher than the ultrasound scanning evaluation of fetal weight in the third quarter and could be slightly improved if it is taken into account along with the ultrasound scanning data. Conclusions: The measurement of the SFH at term may be helpful in foretelling the fetal birth weight and may improve the diagnostic accuracy of the third quarter echographic estimation of birth weight.

Key words: Symphysis-fundal height (SFH); Fetal birth weight; Diagnosis.

Introduction

Already in 1957, Johnson et al. [1] developed a formula for calculating fetal weight starting from the measurement of the height of the uterine fundus. More recently it was reported [2] that the clinical evaluation of the fetal weight starting from such measurement is neither more nor less accurate than the one carried out by ultrasound scanning. Moreover, some articles illustrate the practicality of the measurement of the distance between the pubic symphysis and the fundus of the uterus [3-6], in case an ultrasound scan cannot be executed, while some others suggest that it can be used as a screening method of fetuses with intrauterine growth restriction (IUGR) [7-9]. On the other hand, some authors [6, 10] have also reported that the symphysis-fundal height (SFH) can be used to identify large babies at birth. The aim of this work was to verify the usefulness of the SFH in diagnoses of both low-weight fetuses and large-weight fetuses at birth in full-term pregnancies, and to compare it with the ultrasound estimation of fetal weight, executed at the third quarter echography. Additionally, the clinical effectiveness of symphysis-fundal measurement associated with third quarter echography in predicting birthweight was assessed.

Patients and Methods

The study was carried out in accordance with the ethical standards stated in the Declaration of Helsinki. All patients gave consent for measuring the distance between the symphysis and the fundus of the uterus. Such measurements were carried out on 96 women with single full-term physiological pregnancies and with the fetus in cephalic presentation. The measurements were carried out by means of an inelastic tape graduated in centimeters, from the upper edge of the pubic symphysis up to the higher part of the fundus of the uterus, placing the patient in the gynecological position. Distances were recorded in centimeters and approximated to 0.5 cm. The 3-cm distance of the fetal vertex from an ideal plane passing through the ischial spine and the lower edge of the pubic symphysis was considered as a strict criterion of inclusion. Such distance was evaluated during the obstetric examination, immediately before the symphysis-fundal measurement. Further inclusion criteria were that all the recruited pregnant women had to be submitted to an obstetric ultrasound (US) scan between the 32nd and the 35th week of pregnancy estimating the fetal weight through the Hadlock equation [11], were not obese (pregravidic body mass index less than 29.9), and did not have polyhydramnios or oligohydramnios (excluded with US examination of the amniotic fluid, executed at the time of the symphysis-fundal measurements).

Four classes of fetal weight at birth were arbitrarily considered: < 3,100 g, from 3,100 g - 3,699 g, 3,700 g - 3,999 g, and ≥ 4,000 g. For each of these the diagnostic accuracy (sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), likelihood ratios for a positive and negative test) of the following groups of measurements of the SFH were calculated first: ≤ 33 cm; from 33 cm - 34 cm, and > 34 cm. Then the diagnostic accuracy of fetal growth, expressed in percentiles, estimated according to the US executed in the third quarter was calculated for the following limits: < 50th percentile, in the 50th percentile, > 50th percentile. Finally, the diagnostic accuracy of the measurements of the SFH and the fetal growth estimated according to the US combined together was calculated.
and those weighing 3,100 g - 3,699 g (48 (50%), 3,700 - 3,999 g (16 (16.7%)), borns weighing < 3,100 g resulted to be 17 (17.7%), from 2,550 g and 4,900 g (average 3,520 g, SD 432 g). Newborns weighing 3,100 g - 3,699 g (50%), 3,700 - 3,999 g (16.7%), and those weighing ≥ 4,000 g (15.6%).

Both the Spearman and the Kendall coefficients showed a scanty correlation between the distance from the symphysis to the fundus and fetal birth weight (0.571 and 0.445, p < 0.00001, respectively). They showed an even less significant correlation between the fetal growth estimated according to the US executed in the third quarter and fetal weight at birth (0.434 and 0.327; p < 0.001, respectively).

Table 1 shows the sensitivity, specificity, PPV, NPV and likelihood ratios of the SFH, estimated fetal growth (as regards the 50th percentile) and both for each class of fetal weight considered.

Although the correlation between the SFH and fetal birth weight was stronger in comparison with the estimated parameter of fetal growth, no significant differences of frequency were found in the values of positive and negative predictability, specificity and sensitivity (except for the sensitivities of measurements below 33 cm for fetuses weighing less than 3,100 g). Likewise, when taken together, the SFH and the estimated fetal growth did not significantly improve the diagnostic accuracy. However, values of 33 cm or lower of the SFH in fetuses with growth below the 50th percentile seemed to be more predictive than fetuses whose weight was lower than 3,100 g at birth; SFH between 33 and 34 cm and growth in the 50th percentile seemed to be more predictive than fetuses whose weight was between 3,100 g and 3,699 g; SFH beyond 34 cm and growth above the 50th percentile seemed to be more predictive than fetuses whose weight was higher than or equal to 4,000 g.

Table 1. — Accuracy of the symphysis-fundal height measurements.

<table>
<thead>
<tr>
<th>SFH percentile</th>
<th>&lt; 3,100 g</th>
<th>3,100-3,699 g</th>
<th>3,700-3,999 g</th>
<th>&gt; 4,000 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sens.</td>
<td>82.3%*</td>
<td>17.6%*</td>
<td>31.2%**</td>
<td>4.2%**</td>
</tr>
<tr>
<td>Spec.</td>
<td>78.5%</td>
<td>89.9%</td>
<td>97.4%</td>
<td>66.7%</td>
</tr>
<tr>
<td>PPV</td>
<td>45.2%</td>
<td>27.3%</td>
<td>60%</td>
<td>48.4%</td>
</tr>
<tr>
<td>NPV</td>
<td>95.4%</td>
<td>83.5%</td>
<td>98.7%</td>
<td>49.2%</td>
</tr>
<tr>
<td>LR+</td>
<td>3.8</td>
<td>1.7</td>
<td>6.7</td>
<td>0.9</td>
</tr>
<tr>
<td>LR–</td>
<td>0.2</td>
<td>0.9</td>
<td>0.8</td>
<td>1.0</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>59.5%</td>
<td>79.7%</td>
<td>43.7%</td>
<td>77.1%</td>
</tr>
<tr>
<td>Specificity</td>
<td>68.2%</td>
<td>62.8%</td>
<td>67.6%</td>
<td>95.8%</td>
</tr>
<tr>
<td>PPV</td>
<td>69.4%</td>
<td>56.2%</td>
<td>50%</td>
<td>50%</td>
</tr>
<tr>
<td>LR+</td>
<td>0.3</td>
<td>1.1</td>
<td>1.9</td>
<td>0.4</td>
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<tr>
<td>LR–</td>
<td>1.4</td>
<td>0.8</td>
<td>1.2</td>
<td>0.7</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>5.9%</td>
<td>29.4%</td>
<td>5.9%</td>
<td>25%</td>
</tr>
<tr>
<td>Specificity</td>
<td>59.5%</td>
<td>57%</td>
<td>33.2%</td>
<td>43.7%</td>
</tr>
<tr>
<td>LR+</td>
<td>0.1</td>
<td>0.6</td>
<td>0.6</td>
<td>1.0</td>
</tr>
<tr>
<td>LR–</td>
<td>0.9</td>
<td>1.3</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>95.4%</td>
<td>77.8%</td>
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<td>Specificity</td>
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<td>50%</td>
<td>67.6%</td>
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<td>1.1</td>
<td>1.9</td>
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<tr>
<td>LR–</td>
<td>1.4</td>
<td>0.8</td>
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<tr>
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<td>29.4%</td>
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<td>0.4</td>
</tr>
<tr>
<td>LR–</td>
<td>1.4</td>
<td>0.8</td>
<td>1.2</td>
<td>0.7</td>
</tr>
</tbody>
</table>

*p < 0.035; **p = 0.008.

The values of sensitivity (Sens.), specificity (Spec.), positive predictive value (PPV), negative predictive value (NPV), likelihood ratio for a positive test (LR+) and likelihood ratio for a negative test (LR-) are described for each cutoff of symphysis fundal height, percentile value and both. *p = 0.035 is the level of significance for the comparison between 82.3 % and 17.6 % sensitivity values; **p = 0.008 is the level of significance for the comparison between 31.2 % and 4.2 % sensitivity values. All the other comparisons do not reach significance.

To verify a possible correlation between the distance between the symphysis and the fundus at the fetal weight at birth, and between the third quarter echographic growth and the fetal weight at birth, the Spearman and Kendall correlation coefficients were calculated. Such non parametric tests correct the need of approximation to 0.5 cm of the SFH measurement. Sensitivity, specificity, PPV, NPV and LR were compared through the chi-square test and Fisher’s exact test, considering a minimum cell frequency of significance of p < 0.05.
Usefulness of symphysis-fundal height in predicting fetal weight in healthy term pregnant women

Discussion

The need to know the fetal weight in a single full-term physiological pregnancy seems to be useful only for the management of labor and delivery due to the risk of dystocia or fetal suffering in connection with the fetal weight [12-14]. Usually, in healthy term pregnant women, the fetus weight is not assessed with an echographic scan executed near to the labor date. Therefore, the routine third quarter echographic scan and the clinical evaluation of fetal growth are the only tools to evaluate birth weight. Fetuses weighing less than 2,500 g and more than 4,500 g are very uncommon in a population of healthy pregnant women, thus the usefulness of measuring the distance between the symphysis and the fundus would seem more necessary in the few cases of pregnant women who are not monitored during their pregnancy, in particular with US in the third quarter. In fact, despite the correlation found in this study (similar to the one reported by Bothner et al. [12]), an accurate weight of the baby cannot be obtained from the symphysis-fundal measurement. Moreover, this is in agreement with what was found over 50 years ago by Johnson et al. [1]. We can roughly state that a full-term uterus over 34 cm in length can contain a fetus weighing 4,000 g or more (likelihood ratio 2.4), while a full-term uterus less than 33 cm in length can contain a fetus weighing less than 3,100 g (likelihood ratio 3.8). The first measurement is in accord with what was reported by Winkström et al. [8], while the second is in accord with what was found by Rondó et al. [3]. From a larger case series reported by Walraven et al. [6], we can also understand that the more such cutoffs are increased and decreased, respectively, the more the probability that fetuses with IUGR and macrosomia will be diagnosed.

It may happen that a routine third quarter US scan will find fetal growth under the 50th percentile. Although this is not a pathological finding, it may be a sign of some fetuses that are developing late growth restriction. Since there is no need to repeat the US scan under such conditions, the SFH may be helpful in determining those rare IUGR cases. As far as the results of this study are concerned, the diagnostic accuracy of the SFH is slightly improved when associated with the value in percentiles of the fetal growth estimated by means of the US scan executed in the third quarter, i.e. relating to the low birth-weight fetuses. In any case, there is a fair ratio of large fetuses (3,700 - 3,999 g) which could be more at risk of dystocia and which cannot be easily detected either by US or clinically [14-16].

Barnhard et al. [13] reported that the distance between the symphysis and the fundus can predict a cesarean section for labor arrest. This eventuality can also occur in the absence of very large fetuses. Therefore, in light of what has been reported in this study, it would be interesting to evaluate how much the SFH and US evaluation of the growth together can predict dystocia during labor rather than determining the fetal weight, with a doubtless usefulness for the management of the labor and delivery. A Cochrane systematic review [17], reports that there is not enough evidence to evaluate the use of symphysis-fundal measurement during antenatal care. However, as suggested by this study, SFH at term may be considered as an easy tool to improve the echographically estimated fetal weight between the 32nd and the 35th week of pregnancy, and may be useful in the management of labor.

References


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Mid-trimester maternal serum AFP levels in predicting adverse pregnancy outcome

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Summary

Objective: In this prospective study, we investigated the association between mid-trimester maternal serum alpha-fetoprotein AFP (MSAFP) levels and adverse pregnancy outcome in a South-Western Greek population. Materials and Methods: 110 healthy Greek women with spontaneous pregnancies, investigated for MSAFP levels between the 13th and 24th week of gestation and followed for adverse pregnancy outcome. AFP levels > 2.0 multiples of the median value for gestation were considered abnormal. Statistical analysis was performed by Pearson’s chi-square test. Results: Elevated MSAFP levels were detected in a total of 27 of the 110 women studied (24.5%). Among them, only four women (14.8%) developed pregnancy complications. Conclusion: Multiparameter testing of placental function in the mid-trimester (uterine artery Doppler, placental morphology and MSAFP screening) may allow us to identify women with increased risk of developing severe placental insufficiency and pregnancy complications.

Key words: Maternal serum AFP levels; Adverse pregnancy outcome.

Introduction

Maternal serum alpha fetal protein (MSAFP) was originally introduced for the detection of neural tube defects [1]. However, increased ultrasound machine quality, and sonographer expertise have greatly reduced the need for MSAFP screening in mid-trimester [2].

Pregnancies with unexplained mid-trimester elevation of MSAFP are at increased risk of pregnancy complications [intrauterine growth restriction (IUGR), intrauterine fetal death (IUFD), and preeclampsia (PE)] resulting from placental insufficiency [3-5].

In our prospective study, we investigated the association between mid-trimester MSAFP levels and adverse pregnancy outcome in a South-Western Greek population.

Material and Methods

Between February 2005 and February 2007, about 110 women with spontaneous pregnancies were referred to the Outpatient Clinic of the Obstetrics and Gynaecology Department of the University of Patras Medical School. All women were investigated for MS AFP between the 13th and 24th week of gestation and followed for adverse pregnancy outcome.

Gestational age was estimated from the last menstrual period for women with regular (21-35 days) menstrual cycles or confirmed from ultrasonographic scan in the first trimester for women with irregular menstrual cycles. Women with multiple pregnancies, diabetes mellitus, pregnancy with chromosomal or structural abnormality, hypertension diagnosed before the 20th week of gestation, or history of PE in a previous pregnancy were excluded from the study.

All women had a dated ultrasound examination at their first visit, followed by a detailed examination at the 18th-22nd week of gestation. The study was approved by the Ethical Committee of the Hospital. Written informed consent was obtained from each woman.

Serum samples were collected from all women between the 13th and 24th week of gestation and were stored at -20°C. AFP levels were measured with immunoradiometric assay using two highly specific monoclonal antibodies for coating of the solid phase and the tracer. The tracer antibody and the coated antibody react simultaneously with the AFP present in patient samples or standards. Excess tracer is removed by a washing step and the radioactivity bound to the tube wall is measured in a gamma scintillation counter (IRMA-mat AFP, DiaSorin Inc). MSAFP levels > 2.0 multiples of the median value for gestation (MoM) were considered as abnormal.

Adverse pregnancy outcomes were considered as all gestational complications with fetomaternal circulatory disturbances (PA), IUGR, IUFD, PE.

Placental abruption (PA) was defined as the separation of the placenta from its site of implantation before delivery of the fetus [6].

Intrauterine growth retardation (IUGR) was defined as a birth weight below the 5th percentile for gestational age [7].

Intrauterine death (IUFD) was defined as fetal loss after 24 weeks’ gestation.

Preeclampsia (PE) was defined by a blood pressure above 140/90 mmHg after 20 weeks’ gestation, proteinuria > 300 mg/24 hours or persistent 30 mg/dl (1+ dipstick) in random urine samples. The term severe preeclampsia is used when blood pressure above 160/110 mmHg is recorded at least six hours apart, and proteinuria of more than 5 g during 24 h occurs [8].

Statistical analyses were performed using the SPSS-12 for Windows. The chi-square test was used to assess the association between categoric variables.

Results

Serum samples were collected at a median gestation of 19 weeks (range 13-24). The median weight of the women at the time of serum sampling was 70 kg (range
50-105). The median age at the estimated delivery date was 31 years (range 17-50).

From the 110 women included in the study, ten (9.1%) developed gestational complications during the follow-up of the current pregnancy. The demographic of women with gestational complications compared to those without are shown in Table 1.

Table 1.— Women’s demographics (n=110).

<table>
<thead>
<tr>
<th>No. of pregnancies</th>
<th>Women with complications (n = 10)</th>
<th>Women without complications (n = 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 pregnancy</td>
<td>10 (100%)</td>
<td>85 (85%)</td>
</tr>
<tr>
<td>≥ 2 pregnancies</td>
<td>0 (0%)</td>
<td>15 (15%)</td>
</tr>
<tr>
<td>Age of women &lt; 25</td>
<td>6 (60%)</td>
<td>60 (60%)</td>
</tr>
<tr>
<td>25-35</td>
<td>4 (40%)</td>
<td>23 (23%)</td>
</tr>
<tr>
<td>&gt; 35</td>
<td>2 (20%)</td>
<td>10 (10%)</td>
</tr>
</tbody>
</table>

Abnormal MSAFP levels were detected in a total of 27 of the 110 women studied (24.5%). Among them, only four women (14.8%) developed gestational complications in the current pregnancy. These data are shown in Tables 2 and 3.

Table 2.— MSAFP levels in women with and without gestational complications.

<table>
<thead>
<tr>
<th>MSAFP levels</th>
<th>Women with complications (n = 10)</th>
<th>Women without complications (n = 100)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSAFP &gt; 2 MoM (n = 27)</td>
<td>4</td>
<td>23</td>
<td>ns</td>
</tr>
<tr>
<td>MSAFP ≤ 2 MoM (n = 83)</td>
<td>6</td>
<td>77</td>
<td></td>
</tr>
</tbody>
</table>

p value was calculated by the chi-square test.

Table 3.— MSAFP levels in women with specific gestational complications in the current pregnancy (n = 10).

<table>
<thead>
<tr>
<th>MSAFP levels</th>
<th>PA</th>
<th>IUGR</th>
<th>PE</th>
<th>IUFD</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSAFP &gt; 2 MoM (n = 27)</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>MSAFP ≤ 2 MoM (n = 83)</td>
<td>1</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>3</td>
<td>6</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

PA = placental abruption; IUGR = intrauterine growth restriction; PE = pre-eclampsia; IUFD = intrauterine fetal death.

Discussion

AFP is initially synthesized by the yolk sac, followed shortly thereafter by the fetal liver. Because the human yolk sac involutes at the 9th week, the fetal liver is responsible for most of the AFP production during development [9, 10]. AFP synthesis by the proliferating fetal liver actually increases through the 20th week of gestation, after which it remains fairly constant until the 32nd week [9-11].

Despite the decrease in fetal serum AFP throughout the mid-trimester, MSAFP levels continue to rise until the 32nd week [9-11]. In fact, MSAFP continues to rise well into the third trimester of gestation, with an approximate doubling of maximal values for each trimester [11, 12]. After the 32nd week, MSAFP begins to decline until parturition. Decreasing MSAFP in the third trimester is already related to advancing gestational age [11, 13].

Elevated MSAFP levels have been strongly associated with congenital abnormalities, placental dysfunction and preterm birth [11, 14]. When the fetus is structurally normal, mid-trimester high MSAFP levels are thought to reflect a defect in placentation and are associated with an increased risk of complications in later pregnancy, including severe PE, IUGR and IUFD [11, 14-16]. In our study mid-trimester elevated MSAFP levels were detected in a total of 27 of the 110 women studied (24.5%). Among them, only four women (14.8%) developed pregnancy complications (2 PA, 1 IUGR and 1 IUFD).

In many instances elevated MSAFP levels have been associated with a breakdown in the fetal-maternal placental barrier [17, 18]. It was proposed that an abnormality of the placenta predisposes the pregnant woman to complications, and that this abnormality is initiated early in pregnancy (when MSAFP level is normally measured). Thus, mid-trimester MSAFP was thought to be useful in predicting PE in women who were at high risk for adverse pregnancy outcome and who would require careful monitoring [11].

Hypertensive disorders during pregnancy can reflect pathologic placental conditions that could interfere with the normal passage of AFP to the maternal blood. Thus, the relatively low MSAFP levels during the mid-trimester of pregnancies with hypertensive disorders could be the result of transplacental passage impairment [11]. This observation could aid in the identification of women at risk for such disorders. The apparent paradox was further clarified in a study that found a relationship among MSAFP levels, PE and placental complications [19]. Low MSAFP levels in mid-trimester were found to correlate with a low risk of PE and placental abnormalities, whereas elevated MSAFP levels were associated with a much higher risk for these disorders [19]. However in cases of severe PE, elevated mid-trimester MSAFP levels were always significantly higher than in patients who had mild PE or gestational hypertension [20]. In our study none of the women, developed PE during the current pregnancy.

Placental abnormalities, such as villus lesions, coagulation-related lesions, acute and chronic inflammatory and unclassified lesions, are consistent characteristics of early onset severe PE [21]. The same placental lesions and mainly chronic vascular lesions, such as intervillus thrombosis and chronic villitis, have been described in patients with unexplained mid-trimester high MSAFP levels [21, 22]. This may suggest that early placental pathology permits a more rapid diffusion of AFP from the fetoplacental compartment to the maternal compartment [22].
Elevated MSAFP in mid-trimester has been shown to be associated with a 2.3- to 3.8-fold increased risk of developing PE [23, 24]. In our study none of the women with mid-trimester elevated MSAFP levels developed PE during the current pregnancy.

Recent studies have shown that MSAFP levels at the 22nd-24th week were not significantly different in cases during the current pregnancy.

with mid-trimester elevated MSAFP levels developed PE [23, 24]. In our study none of the women developing PE [23, 24]. In our study none of the women 

References
tal insufficiency and pregnancy complications. 
ogy and MSAFP screening) may allow us to identify 

cant placental pathology leading to PE and IUGR [15, 26].

Conclusion

Multiparameter testing of placental function in the mid-trimester (uterine artery Doppler, placental morphology and MSAFP screening) may allow us to identify women with increased risk of developing severe placental insufficiency and pregnancy complications.


tal insufficiency and pregnancy complications.

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Knowledge and general consideration about Pap test screening among women from Finland and Greece

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Summary

It seems that lack of regular Pap smear screening is a risk factor for cervical cancer. Since women started having Pap smears (more than 50 years ago), the number of deaths from cervical cancer has dropped dramatically. The purpose of this study was to investigate the knowledge of women about the essential and basic parameters related to the Pap test in the general population of two European countries: Finland and Greece. The same percentages (> 50%) of women in Athens and Helsinki had good knowledge of the Pap test. Comparing country populations, greater numbers of Finnish women had a better level of Pap test knowledge. Additionally, older women in Finland were more educated and informed about the usage of Pap testing due to the good health service information in this country.

Key words: Pap Test; Cervical cancer screening; HPV.

Introduction

It seems that lack of regular Pap smear screening is a risk factor for cervical cancer. Since women started having Pap smears (more than 50 years ago), the number of deaths from cervical cancer has dropped dramatically. The chances of cure are 100% for cervical cancer stage 0 (in situ), and as high as 90% if cervical cancer is discovered early on (stage IA-IB).

Human papillomavirus (HPV) infection is the most common cause and a major risk factor for the development of cervical cancer. There is no evidence that herpes simplex virus (HSV) infection can result in cervical cancer. There are more than 100 subtypes of HPV that infect the genital tract: vulva, vagina, cervix, anus and penis, causing warts and dysplasias. The subtypes 16 and 18 are the most frequently associated with cervical cancer, and are in contrast with subtypes 6 and 11 which are associated with "condylomata acuminate". Women who do not regularly have a Pap smear to detect HPVs or abnormal cells in the cervix are at increased risk of cervical cancer. The HPV effect can be expressed as koilocytes in superficial or intermediate squamous cells, or sharply delineated perinuclear halos in parabasal cells. Occasionally, binuclear cells are present in cases of reactive squamous atypia (which rarely is associated with HPV infection). The importance of HPV detection is emphasized by the fact that multiple, large, well-controlled screening trials have clearly demonstrated that HPV testing is considerably more sensitive than cytology, and only slightly less specific when used in women 30 years of age and older [1].

Although the Pap test is the best tool for detecting pre-cancerous changes of the uterine cervix and cervical cancer in its earliest stage, the conventional Pap test has a considerable rate of false negatives. The published range of false negatives is 1.6-28% [2]. In order to improve the efficiency and sensitivity of the Pap test and reduce screening time, several types of technologies were developed, including computer technology (Papnet), and liquid-based cytology [3]. Comparing liquid-based cytology versus the conventional Papanicolaou smear, it seems that the first can improve high-standard cervical cancer screening cytology even further.

Liquid-based techniques are used routinely in many countries, and ThinPrep tests have achieved an increase in sensitivity, a dramatic improvement in specimen adequacy, and a reduction in screening time [4].

The importance of women’s knowledge regarding Pap testing (including some basic knowledge regarding HPV and diagnosis) is recognised by very recent studies [5, 6]. The lack of regular Pap smear screening is related to inadequate information about the Pap test [5]. On the contrary, the probability of having regular Pap tests is greater in women who know that lack of Pap testing increases the risk of cervical cancer [6]. The purpose of this study was to investigate the knowledge of women about the essential and basic parameters related to the Pap test in the general population of two European countries: Finland and Greece.

Population and Methods

The study sample consisted of 400 women of different ages and educational levels from Greece and Finland. Two hundred women were from the capital (100 from Athens and 100 from Helsinki), and 200 women were from the province (100 from Kalymnos island, Greece and 100 from Kuopio, Finland). To estimate the knowledge of pap testing in the above sample, all women completed a questionnaire consisting of 12 multiple-choice questions.
The questions were as follows:

Do you know what a Pap test is?
- Yes
- No
- Partially

Have you ever been informed about the Pap test? If yes, where from?
- Primary school, high school
- Friends, relatives
- Books, magazines
- Mass media
- Other way

Why did you have a Pap test for the first time?
- Prevention
- Doctors advice
- Friends and family advice
- Pregnancy
- Population screening

How often do you have a Pap test?
- Annually
- Every 2 years
- Every 4 years
- Rarely
- Never

If you rarely have a Pap test, what is the reason?
- Economical
- Fear
- Embarrassment
- Negligence
- Lack of health structure

Until what age must women have a Pap test?
- Until 50
- Until 70
- The end of life
- I don’t know

How many types of the Pap test are you aware of?
- Classic
- Liquid-based
- Classic+liquid based
- I don’t know

What is the percentage of false-negative results of the test?
- If a Pap test is abnormal, what exams (and/or therapy) should you have?
- Colposcopy-biopsy
- Curettage
- Hysterecctomy
- Radiation
- I don’t know.

If you have a Pap test and also colposcopy in situ malformations, what is the percentage that can be cured?

Is there someone in your immediate family with cervix cancer?
- Yes
- No
- I don’t know.

Results

The same percentages (57%) of women in Athens and Helsinki had good knowledge of Pap testing. Comparing country populations, greater numbers of Finnish women had a better level of Pap test knowledge. The latter difference is attributed to better knowledge of the Pap test in provincial Finish women compared to those in Greece. In Finland, the women’s answers were almost the same in the capital (57% with correct knowledge and 20% ignorance) and in the rural areas (53% with correct knowledge and 16% ignorance). However, in Kalymnos and Kuopio, the percentages were 39% and 19%, respectively. The rest of the women in both countries knew partly what the Pap test is. Only 117 Greek women knew both methods of the Pap test (conventional and liquid), while 144 Finnish women had that knowledge (p = 0.04). Similarly, only 54 women from Kalymnos had that knowledge, while the corresponding number in Kuopio was 75 (p = 0.002).

In the age group under 20, lower percentages of women had a good knowledge of Pap testing in both countries (35% in Athens, 30% in Kalymnos, 20% in Helsinki and 15% in Kuopio). In contrast, groups with higher ages had a better knowledge of Pap testing in both countries. In the age group > 36 (36 to 50), a better knowledge could be seen in Finnish women from Helsinki (82%) compared to those from Athens (68%), while there was no difference comparing women from Kuopio to those from Kalymnos (68% and 66%, respectively). This fact can be attributed to the higher educational level of Finnish women from the capital. The same conclusion could be drawn for older women (over 65), where a better knowledge could be seen in Finnish women from Helsinki (75%) compared to those from Athens (40%).

The doctor was the main information source in relation to the others (school, friends, books, mass media). This was the same in both countries. The main difference was that school was the second source in Finland whereas in Greece it was mass media.

The two main reasons why women do the Pap test for the first time were prevention and doctors’ advice. In Greece and particularly in Athens the main reason was the doctor’s advice (about 60%). In the island of Kalymnos this percentage was about 44%. In Finland, in Helsinki and Kuopio, these percentages were 57% and 64%, respectively. The difference between Kuopio and Kalymnos could be related to screening policies in Finland.

In contrast, in Greece there is no population screening. The question as to how often do you have a Pap test, a great percentage of Athenian women (46%) have the test every year in contrast to women who live on Kalymnos island, of which 40% have never had the test. The latter percentage in Kuopio was only 20%. In Finland most women in Helsinki and Kuopio are checked every four years. This can be attributed to mass screening which is obligatory for women over 40 every four years.

The percentage of the women who had never had a Pap test in relation to age was very high for ages under 20 (in Athens and Helsinki: 65% and 100%, respectively, and in Kalymnos and Kuopio 90% and 65%, respectively). It should be noted that according to recent standards, Pap testing should not be done in ages under 20, where a great percentage of HPV infection may be found, but with high rates of remission. The test should not be offered to women under 20 because it could result in the over-treatment of many cases. This kind of policy was found in Helsinki.

Fear (80%) and shame (90%) were the two main reasons that the Kalymnian women avoided Pap testing. In contrast the women of Kuopio had Pap tests systematically and if not it was because of ignorance (10%). In Athens, the main reason was economic (40%) but in Helsinki it was because of ignorance (60%).

Many women from both countries were not sure until what age they should have Pap tests (in Athens and Helsinki, 54% and 52%, and in Kalymnos and Kuopio 41% and 65%, respectively). Some gynecologists suggest that the test should be performed until the age of 70 because the chances of disease are very low after age 70. We will not further validate these results because there are no universal policies for the age of stopping Pap smears (range 50 to 64 years in Europe countries).

In Athens 14% of the women knew about the new liquid-based Pap test whereas 1% in Helsinki knew. However, in Kalymnos and Kuopio there was no difference (2% and 1%).

With Pap testing vaginal and cervical inflammations caused by viruses (herpes simplex virus, HPV), bacteria, and germs can be diagnosed. Although most Greek
women were aware that with a Pap smear, infection-inflammation could be diagnosed, these results will not be further evaluated because the main purpose of the test is to diagnose cervical dyskaryosis (corresponding to intraepithelial lesions).

The percentages of false-negative results of the test were 10-30%. In Athens most of the women (56%) knew the right answer but a great number of Finnish women in Helsinki (70%) did not. In Kalymnos and Kuopio, there was no significant difference (30% and 34%, respectively). More Athenian women (n = 57) compared to women from Helsinki (n = 41) (p = 0.02), knew that colposcopy, with or without biopsy could be the next diagnostic step after a non-normal Pap test (with HPV determination as an alternative in ASCUS). Dysplasia can be cured in 100% of cases (and the diagnosis is the main purpose of Pap testing). Less than 27% from both countries were aware of this. They believed that dysplasias could be cured in only about 50% of cases.

Discussion
This study has investigated the general knowledge of women about Pap screening from two different European countries, Greece and Finland. The sample included women from the two capitals of Athens and Helsinki, and two provincial cities, Kalymnos and Kuopio. Results demonstrated that Greek and Finnish women originating from the capital had a better knowledge about Pap testing compared to women from the provinces.

In a study by Tiro et al. [7], among women who had heard about HPV, a great percentage did not know that it causes cervical cancer and that HPV is sexually transmitted and causes abnormal Pap tests. The factors associated with having heard about HPV included: younger age, higher educational, exposure to multiple health information sources, trusting health information, regular Pap tests, awareness of changes in cervical cancer screening guidelines, and having tested positive for HPV [7]. Knowledge about screening was related to exposure to multiple health information and screening guideline centers. Similarly, in Finland, due to multiple health information, older women had a better knowledge compared to Greek women. This fact is also supported by the results of general Pap screening in Finland which has led to an age-standardized incidence rate of 4/100,000 and mortality rate of 1/100,000 women [8]. Income and education are considered to be better predictors of knowledge about cervical cancer prevention than rural residence. Higher rates of cervical cancer in rural areas may reflect lower education and lower income [9]. The greater ignorance of Pap testing in women of Kuopio and Kalymnos (rural parts of the two countries) could be attributed to lower economic standards and educational levels of these regions compared to women from the capital cities.

In a Brazilian study, reflecting the main reasons for not having a Pap smear test done before, were embarrassment and fear of pain in most of the cases. In this study knowledge of HPV infection and cervical cancer was low in this young (urban) population [10]. Results in our study have shown that older women between 36-50 years knew more about Pap testing compared with those under 20 years of age.

Another basic theme was related to the lack of a physician recommendation which contributes to under-use of Pap screening by many eligible women.

Research shows the effectiveness of physician recommendations in improving the use of Pap testing [11]. Increased physician recommendations for Pap tests were noted in Greece and Finland, suggesting the pivotal role of the doctor in screening examinations. Different approaches may be effective in reducing the psychosocial access that contributes to cancer health disparities in underserved populations [12]. Liquid-based cytology is a new vanguard method with high accuracy and sensitivity but with increased cost in the National Health System [13]. Greek women in urban areas with higher economic status have a better knowledge of this new method compared to those located in rural districts with lower economic incomes, like Kalymnos. Reminder services may be a cost-effective way to increase Pap test rescreening rates. These services must follow any changes in the personal data of the women screened (address, phone, etc.) [14]. Data from the literature have suggested that the basic three determinants of social inequity in healthcare service usage are social class, gender, and race or ethnicity [10]. Concerning the last determinant, we have seen in our study that older women in Finland are more educated and informed about the use of Pap testing due to the very good health service information system in this country.

Conclusion
It is universally accepted that Pap screening has dramatically decreased the rate of cervical cancer.

The general knowledge of women about this inexpensive and simple method depends on several factors. Older women are more informed than younger women. Female populations in urban districts have a wider knowledge and clearer orientation about the purpose of the examination. National Pap test screening may prevent cervical cancer spread.

Healthcare services should always offer extensive information about the aim of the Pap test to all women regardless of their social and economic status.

References


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Pregnancy and delivery following sonohysterographic lysis to treat recurrence after hysteroscopic lysis of severe intrauterine adhesions: a case report

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Summary

Objective: To report a patient with sterility secondary to severe intrauterine adhesions who underwent sonohysterographic (SHG) lysis for recurrent adhesions following hysteroscopic lysis, and achieved tubal patency and natural pregnancy leading to term vaginal delivery. Design: Case report. Setting: National Hospital Organization Kyoto Medical Center, Kyoto, Japan. Patient: A patient with hypomenorrhea and sterility due to postpartum severe intrauterine adhesions. Interventions: Operative hysteroscopy was performed for the severe intrauterine adhesions, and SHG lysis was performed for each of the recurrent adhesions that had occurred four times. Results: SHG lysis improved the hypomenorrhea and restored the patency of the occluded fallopian tube. The patient became pregnant, and vaginally delivered a full-term infant. Conclusion: This approach may be an option if recurrent adhesions following hysteroscopic lysis occur.

Key words: Intrauterine adhesions; Operative hysteroscopy; Pregnancy; Sonohysterography.

Introduction

Intrauterine adhesions frequently develop after intrauterine procedures, such as dilatation and curettage, postpartum management, and hysteroscopic myomectomy, and cause menstrual disorders such as amenorrhea or hypomenorrhea, leading to sterility or infertility [1-3]. Although different classifications have been reported, intrauterine adhesions are broadly classified into mild, moderate, and severe categories [4-6]. Severe intrauterine adhesions account for 9.3-25.1% of the total cases [6-8] and usually are treated by hysteroscopic lysis. However, even if severe adhesions are lysed, recurrent adhesions frequently occur [6, 7, 9]. In performing sonohysterographic (SHG) lysis, Coccia et al. achieved good results for mild adhesions [10]. However, few reports have examined SHG lysis of recurrent adhesions following hysteroscopic lysis of severe intrauterine adhesion. We describe a patient with severe intrauterine adhesions who underwent hysteroscopic lysis and SHG lysis of recurrent adhesions, and afterward achieved tubal patency, pregnancy, and term delivery.

Case Report

A 37-year-old gravida 1, para 1 woman had a fever of more than 38°C, and a retained placenta after the vaginal delivery of her first child in a hospital, and underwent intrauterine curettage. Since that time, she had had regular but light menstrual periods (approximately one-seventh of the pre-pregnancy amount of menstrual flow), and failed to become pregnant despite her wishes to have a second child; thus, she was visited at Kyoto Medical Center. She had regular menstrual cycles and normal levels of follicle stimulating hormone, luteinizing hormone, prolactin, and estradiol. Only the left side of the uterine cavity and the left fallopian tube were visualized by hysterosalpingography (HSG), and the patient was diagnosed with intrauterine adhesions (Figure A). On the day before surgery, a 2-mm in diameter laminaria (dried kelp stalk) was inserted into the cervical canal, and gauze was placed in the vagina. After 15 hours, they were removed under spinal anesthesia. After dilatation of the cervical canal with Hegar dilators, operative hysteroscopy was performed using a resectoscope and Uromatics (d-sorbitol; Baxter, Tokyo, Japan) as distending medium. Operative hysteroscopy was performed, with findings categorizing the intrauterine adhesions as severe according to the American Fertility Society Classification of Intrauterine Adhesions [4]. The adhesions were incised with a rigid resectoscope with a hook-shaped monopolar electrode, using transabdominal ultrasonographic guidance. The uterine opening of the right fallopian tube could not be identified. Surgery was completed in 35 min without perioperative or postoperative complications. With the introduction of anesthesia, flomoxef sodium (Shionogi & Co., Ltd., Osaka, Japan) as a prophylactic antibiotic was administered at a dose of 1 g, followed by another dose eight hours after surgery. The patient was discharged 24 hr after surgery. An intrauterine device (IUD) was inserted into the uterine cavity, and estrogen (E) and progesterone (P) therapy was administered for three months. After three withdrawal bleedings, the IUD was removed. HSG clearly visualized the entire uterine cavity.

We have participated in the work take responsibility for the manuscript which has never been published or submitted for publication elsewhere.

No financial support has been received.

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and the left fallopian tube, but failed to visualize the right fallopian tube (Figure B). The amount of menstrual flow returned to normal after E and P therapy, but then declined by half at eight months after surgery, suggesting adhesion recurrence. We explained the need for hysteroscopic confirmation of recurrence and lysis to the patient, who declined to undergo repeat surgery by hysteroscopy. Instead, SHG was performed to diagnose and treat recurrent adhesions in the outpatient clinic. After an 8-Fr uterine injector catheter (Hyscath; Sumitomo Bakelite, Tokyo, Japan) was inserted into the cervical canal, the balloon at its tip was filled with 0.5-1.0 ml of physiological saline, and fixed in an appropriate position. Without anesthesia and during trans-vaginal ultrasonographic monitoring, approximately 7-10 ml of physiological saline was injected into the uterine cavity, and intrauterine adhesions were confirmed [11]. The saline was aspirated, followed by lysis by two to three rapid injections of 10-12 ml of physiological saline under moderate pressure. The procedure was concluded after confirming that the uterine cavity was fully expanded. Subsequent menstrual flow volumes returned to normal, but decreased again at 10, 13 and 15 months after hysteroscopic surgery. After each decline SHG lysis was repeated with restoration of normal menstrual flow. After the second menstrual flow decrease ten months after the first hysteroscopic surgery, HSG showed adhesions in the right uterine cavity but visualized the previously occluded right fallopian tube (Figure C). The patient became pregnant naturally 18 months after surgery. The course of the pregnancy was uneventful, including vaginal delivery of a healthy infant at 37 weeks of gestation. Although placental adhesions were observed at childbirth, manual removal of the placenta was successful.

**Discussion**

Lysis of severe intrauterine adhesions is frequently followed by recurrent adhesions [6, 7, 9]. Patients with such recurrences often undergo hysteroscopic lysis several times after the initial lysis. Pabuccu et al. [7] observed recurrent adhesions in six (60%) of ten patients. Cappella-Allouc et al. [9] noted filmy adhesions in ten out of 31 patients developing recurrent adhesions after lysis of severe intrauterine adhesions, and reported successful hysteroscopic lysis without anesthesia on an outpatient basis, but 15 patients with mild or severe adhesions were hospitalized, and underwent hysteroscopic lysis under anesthesia: seven patients required lysis twice, seven patients three times, and one patient four times. Valle et al. [6] observed recurrent adhesions in 23 of 47 patients with severe intrauterine adhesions, and recurrent adhesions in seven of 20 patients who underwent lysis of recurrent adhesions. In some patients, recurrent adhesions are more severe than at the time of initial surgery. These suggested a high possibility of recurrent adhesions in patients with severe intrauterine adhesions. Our patient required SHG lysis for recurrent adhesions four times. As SHG lysis was performed promptly when menstrual volume decreased, SHG lysis may be effective when carried out before adhesions become fully established.

Some authors have reported restoration of tubal patency by initial hysteroscopic lysis of severe intrauterine adhesions [12], but such a result is very difficult to obtain. Even if the initial lysis failed to achieve tubal patency, SHG lysis after the hysteroscopic lysis restored

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**Figure (A)** Preoperative hysterosalpingogram (HSG) showing patency of a small left portion of the uterine cavity and left fallopian tube. (B) HSG performed three months after surgery showing patency of the uterus and left fallopian tube. (C) HSG performed ten months after surgery showing constriction of the right portion of the uterine cavity and patency of both fallopian tubes (after one treatment with sonohysterographic lysis).
Pregnancy and delivery following sonohysterographic lysis to treat recurrence after hysteroscopic lysis of severe intrauterine etc.

Previous studies of severe intrauterine adhesions have reported varying results of lysis in terms of pregnancy and delivery. Pregnancy and live birth rates have been 0-57.4% and 0-32.1%, respectively [6-8]. Pabuccu et al. reported that sterile patients with severe intrauterine adhesions did not become pregnant [7]. Capella-Allouc et al. observed pregnancy in 12 of 28 patients, and nine of these 12 patients gave live birth, with four having vaginal delivery of term infants [9]. Valle et al. reported that 13 of 30 sterile patients achieved pregnancy, including five with term pregnancy [6]. These data suggest that it is not so easy to achieve term delivery when a severe intrauterine adhesion is recognized. We have observed severe intrauterine adhesions only in the present patient, who underwent SHG lysis for recurrence four times, with improvement in tubal occlusion. Later, the patient had natural menstrual cycles, became pregnant, and had vaginal delivery of a live term infant. SHG lysis typically can be performed without anesthesia on an outpatient basis for diagnosis of intrauterine adhesions [11], lysis of intrauterine adhesions, and restoration of tubal patency. Disadvantages include inability to fully confirm the location and status (mild, moderate, and severe) of adhesions, and likely inability to lyse some severe recurrent adhesions. SHG lysis thus, may be an option when decreased menstrual flow suggests recurrence of adhesions, and when initial lysis fails to restore tubal patency.

References


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A case of body stalk anomaly at 12 weeks of gestation

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Summary

We report a case of body stalk anomaly which was diagnosed at 12 weeks of gestation on a first trimester scan. The fetus displayed multiple anomalies characteristic of body stalk syndrome including abdominal wall defect, kyphoscoliosis, deformities of the lower limbs and a single umbilical artery. Body stalk anomaly is a rare congenital anomaly with a series of similar clinical manifestations and poor prognosis. The first trimester scan can estimate the risk for chromosomal abnormalities and may also reveal major congenital abnormalities.

Key words: Body stalk anomaly; Ultrasound; Early pregnancy; First trimester scan.

Introduction

Body stalk anomaly (BSA) or limb-body wall complex (LBWC) is a severe congenital anomaly with a series of similar clinical manifestations and poor prognosis [1]. It describes a typical pattern of defects that include encephalocele, facial cleft, an anterior abdominal wall defect, kyphoscoliosis, limb deformities and an absent or short monoarterial umbilical cord. Its prevalence has increased from approximately one in 14,000 pregnancies [2] to one in 7,500 in recent studies [3]. This increase could be explained by the widespread use of first trimester ultrasound which enables a diagnosis to be made in cases which were destined to end up in spontaneous miscarriage [4]. A variety of hypotheses have been proposed to explain the pathogenesis of BSA, including mechanical damage due to early amniotic rupture, vascular disruption to the early embryo and teratogenic exposure in early pregnancy. An early amnion rupture before obliteration of the coelomic cavity has been discussed to be the major cause of this anomaly. The extra embryonic coelom fails to obliterate and parts of the fetal body remain in an exocoelomic situation. The intraabdominal content persists in the extra-embryonic coelom in a sac covered by amnion and placenta [3]. Van Allen et al. [1] found the anomalies to be secondary to vascular disruption at an early stage of gestation. In this context, an association of BSA to maternal drug abuse, especially cocaine has been described [5].

We report a case of body stalk anomaly which was diagnosed at 12 weeks of gestation on a first trimester scan.

Case Report

A 37-year-old primigravid woman at 11+6 weeks of gestation was referred for the first trimester scan. She had had an episode of bleeding from subchorionic hematoma during the 9th week of gestation (Figure 1) and was taking natural microionized progesterone at a dose of 300 mg daily/po. There was no relevant medical history and she was taking no other medication.

Ultrasound examination revealed an intrauterine gestational sac. The sac contained a live fetus with a crown-rump length (CRL) of 47.3 mm, consistent with a gestational age of 11 weeks and 4 days (Figure 2). The nuchal translucency was normal (2.1 mm). The amniotic membrane appeared normal and intact. The fetus was seen to be in the exocelomic cavity and there were multiple abnormalities. The combination of defects comprising an anterior abdominal wall defect containing liver and bowel, kyphoscoliosis, deformities of the lower limbs and a single umbilical artery (Figure 3), were in accordance with the diagnosis of body stalk anomaly.

The parents were counseled that this is a lethal condition for the fetus. They chose a surgical termination of the pregnancy, so an uncomplicated procedure was performed under general anesthesia the next day. The specimen which was obtained at the procedure was disrupted and a detailed pathologic examination was impossible.

Discussion

A heterogeneous group of congenital defects has been referred to as BSA or LBWC. This case demonstrates typical features of body stalk anomaly in a fetus which was developing in the exocelomic cavity. These findings are in accordance with the theory that early amniotic rupture with expulsion of the embryo into the exocelomic cavity is responsible for the features of BSA. The rupture allows part of the fetal body to pass to the coelomic cavity leading to structural defects, mainly of the abdominal wall and spine. The formation of amniotic bands can produce limb deformities. Abnormal development at the trilaminar stage would account for the common finding of a single umbilical artery, which is present in over 50% [1] of the cases, while in the general population it is less than 1%.

Many authors have discussed possible etiologies of these anomalies including teratogens [6], an amniotic band or rupture sequence [7, 8], intrauterine compression [7], failure in morphogenesis [8], a vascular defect [1], placental insufficiency [9] and multifactorial etiology [10].
A case of body stalk anomaly at 12 weeks of gestation

An explanation of BSA is the theory of vascular disruption during the first four to six weeks of gestation developed by Van Allen et al. [1]. They have defined the concept of LBWC as a combination of at least two of the following characteristics: 1) Exencephaly or encephalocele with/without facial clefts, 2) thoracic and/or abdominal ventral body wall defects, and 3) limb defects. Colpaert et al. [10] have argued that at least two different types of LBWC can be distinguished from each other. The craniofascial defect type (LBWC type 1), is characterized by abnormal attachment of amnion, which may cause cranial or fascial disruption and/or enencephaly or encephalocele combined with limb defects and/or abdominal wall defects. It is well-explained by an amniotic band or rupture sequence, or a compression theory. The ventral body wall defect type, BSA type or LBWC type 2, is characterized by an umbilical cord anomaly (short, single umbilical artery), thoracic and/or abdominal ventral wall defects with or without limb defects and scoliosis, but does not present with craniofascial defects.

BSA is one of the possible causes when an increased nuchal translucency is observed during the 1st trimester scan [3, 11]. In conclusion obstetricians, neonatologists and pediatric surgeons must recognize these malformations as lethal and counsel parents to make an informed decision regarding the pregnancy.

References


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Partial placenta increta and methotrexate therapy: three case reports

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Summary

The term placenta accreta is used to describe any placental implantation in which there is abnormally firm adherence to the uterine wall. This condition complicates 1/2,500 deliveries and is rising in incidence. Abnormal placentation is associated with increased maternal morbidity and mortality from severe hemorrhage, uterine perforation, infection and loss of fertility. The reported experience of methotrexate treatment in the conservative management of placenta accreta is scant. Three cases of partial placenta increta managed with methotrexate are described. The patients were assessed with clinical surveillance, serum β human chorionic gonadotrophin (β-hCG) and imaging (ultrasonography and magnetic resonance in one case). In all cases conservative management with methotrexate resulted in undetectable serum β-hCG, a decrease in the size of partial placenta retained, and undetectable vascularization.

Key words: Placenta accreta; Conservative management; Methotrexate.

Introduction

The term placenta accreta is used to describe any placental implantation in which there is abnormally firm adherence to the uterine wall. There are three varieties of placenta accreta: placenta accreta vera if chorionic villi are attached to the myometrium; placenta increta in which the chorionic villi invade the myometrium; placenta percreta in which the whole thickness of the myometrium is invaded to the serosal surface with possibility of rupture into the peritoneal cavity. The abnormal adherence may involve all, a few or a single cotyledone (total, partial and focal). The condition complicates 1/2,500 deliveries and is rising in incidence. Associated risk factors include manual removal of placenta at a previous birth, vigorous and repeated curettage, presence of submuocos fibroids, placenta previa, pregnancy in the uterine diverticulum and previous cesarean section scar. Abnormal placentation is associated with increased maternal morbidity and mortality from severe hemorrhage, uterine perforation, infection, and loss of fertility.

The reported experience of methotrexate treatment in the conservative management of placenta accreta is scant, based on few reported cases. Three cases of partial placenta increta managed with methotrexate are presented. The patients were followed-up with clinical surveillance, serum β-hCG and imaging (ultrasonography and magnetic resonance in one case). In all cases conservative management with methotrexate resulted in undetectable serum β-hCG, a decrease in the size of partial placenta retained, and undetectable vascularization.

Case Reports

Case 1

A 27-year-old woman was admitted to the delivery ward in premature labor at 26 weeks and five days of gestation. She had had a previous termination of pregnancy at 14 weeks with curettage, and there was no significant previous medical history. Two antenatal ultrasound (US) examinations showed a healthy fetus and an anterior fundal placenta. Cesarean section under epidural analgesia was performed because of breech presentation, and a male preterm infant, weighing 970 g was delivered. The placenta was retained and an examination in the operating room through the uterine incision confirmed that a portion of the placenta was firmly adherent to the right horn of the uterus, which was left there. During surgery there was heavy bleeding that was controlled with intravenous oxytocine, and a transfusion of two units of blood (hemoglobin 7.6 g/dl). The patient consented to be treated with 70 mg of methotrexate (50 mg/m²) intramuscularly at first and eight days postpartum. On the 8th postpartum day Doppler US showed that the placental mass was in the right horn of the uterus, invading the myometrium, with increased vascularity and measuring 50 x 45 mm. The patient remained well and serum β-hCG was undetectable on postpartum day 17. The following Doppler US on postpartum day 24 showed no vascularity and a slight decrease in the placental mass (49 x 42 mm) (Figure 1). Five months postpartum, a further US showed that the mass measured 33 x 21 mm (Figure 2). Eight months following delivery, the patient had a normal pelvic ultrasound (Figure 3).

Case 2

A 33-year-old woman was admitted at 22 weeks of gestation because of ruptured membranes. She had a previous termination of pregnancy at seven weeks without curettage, and there was no significant previous medical history. She progressed quickly to full dilatation, and after fetal expulsion the placenta was retained and an attempt to remove all placental tissue manually was unsuccessful, so she was submitted to curettage under US control. Doppler US confirmed a portion of retained placenta in
the right horn, measuring 40 x 40 mm, up to 8 mm from the serosa. Because the uterus was well contracted, and there was no active bleeding, the procedure was finished and intravenous oxytocine, antibiotics and rectal misoprostol were administered. On postpartum day 4, 70 mg of methotrexate (50 mg/m²) intramuscularly were given (after consent). On postpartum day 9, Doppler US showed that the placental mass was in the right horn of the uterus, invading the myometrium, with increased vascularity and measuring 33.3 x 32.8 mm (Figures 4 and 5). The patient remained well and serum β-hCG was undetectable on the 23rd postpartum day. A follow-up Doppler US one month after delivery still showed vascularity but a decrease in the placental mass (25.6 x 23.1 mm). Two months later, the mass measured 20 x 20 mm and vascularity was undetectable. Eleven months following delivery an US revealed a calcified mass of 15 x 9 mm (Figure 6). The patient feels well, menses are regular and with normal flow, and she plans future pregnancies.

Case 3
A 37-year-old woman was admitted at 32 weeks of gestation because of ruptured membranes. She had had two previous terminations of pregnancy with curettage, and a previous cesarean section because of face presentation. At 34 weeks, because of breech presentation, an elective cesarean section was performed under epidural analgesia with the delivery of a preterm male infant, weighing 2,280 g. The placenta was partially removed and an examination in the operating room during surgery, through the uterine incision, confirmed that a portion of the placenta was firmly adherent to the left horn of the uterus. Because there was not any substantial bleeding and the uterus was well contracted, the patient was treated with intravenous oxytocine, rectal misoprostol and antibiotics. Doppler ultrasound on postpartum day 4 confirmed a portion of retained placenta in the left horn, measuring 69 x 50 mm up to the serosa, with increased vascularity (Figure 7). She consented to two administrations of 82 mg of methotrexate (50 mg/m²) intramuscularly on that same day and on the 11th postpartum day. MRI was performed on the 13th day after delivery to exclude placenta percreta. The images showed an enlarged uterus with a portion of placental tissue, but with no evidence of placenta percreta (Figure 8). The patient had no abnormal bleeding and serum β-hCG was undetectable 27 days after delivery. A follow-up Doppler US one month postpartum showed undetectable vascularity and a decrease in the placental mass (37 x 36 mm). Three months later the mass measured 24 x 22 mm (Figure 9). The patient is feeling well and does not plan any future pregnancies.

Discussion
Placenta accreta is a rare condition associated with considerable maternal morbidity and mortality. The classic review of Fox [1] in 1972 suggested a hysterectomy in women with placenta accreta to avoid serious complications. In an attempt to preserve childbearing function, a conservative mode of treatment with methotrexate has been described in a few cases. Arulkumaran et al. [2] in 1986 first reported the successful treatment of placenta accreta with methotrexate. They used 250 mg of intravenous methotrexate (5 doses on alternate days from postpartum day 1); expulsion of a necrotic placenta occurred on the 11th day of treatment. In 1990, Hwang JL et al. [3] described an unsuccessful case of conservative management of placenta increta. They used three doses of methotrexate (not mentioning the dose and route of administration) which required hysterectomy because of persistent vaginal bleeding. Raziel et al. [4] reported a case of placenta accreta treated with two doses of 20 mg of methotrexate IM, on the fourth and fifth postpartum day, which resulted in expulsion of a necrotic placenta on postpartum day 7. In the report by Legro et al. [5], in a case of placenta percreta they used ten doses (1 mg/kg per week) of intramuscular methotrexate, and the outcome was a slow involution of placenta over five to six months postpartum. In the same year Jaffe et al. [6] reported a case of correct antenatal diagnosis of placenta percreta invading the bladder wall. Their patient underwent cesarean section and the placenta was left in situ. She had 50 mg of methotrexate/week for six weeks (route of administration not specified). Because of vaginal bleeding a hysterectomy with partial cystectomy and ureteric reimplantation was necessary. More recently four more articles with case reports have been published. Bucksee et al. [7] presented a case of placenta accreta treated with 50 mg of intravenous methotrexate (3 doses) with expulsion of placental tissue on the 18th postpartum day. In 2000 Mussali et al. [8] presented three cases of placenta accreta treated with methotrexate which resulted in uterine preservation in two of the cases. Panoskaltsis et al. [9] in the same year presented two cases of placenta increta, one treated expectantly and the other with methotrexate; neither required hysterectomy. Nijman et al. [10] reported a case of placenta percreta treated successfully with conservative management with methotrexate IM weekly (1 mg/kg) in a total dose of 240 mg.

Based on the few reported cases and our three recent cases it has been demonstrated that conservative treatment of placenta accreta can be successful. In our three cases, we did not leave the entire placenta in situ; a portion of the placenta was manually removed and we left in situ just a portion of placenta (partial increta placenta). We decided to start treatment with methotrexate because of persistent partially retained placenta with continuing active vascularity. The route of administration was intramuscular and the dose used was the same that we use for ectopic pregnancy (50 mg/m²). It unknown whether a successful outcome has any relation to the amount of retained tissue, degree of placenta accretia, the dose used or other factors. The patients were followed-up with clinical surveillance, serum β-hCG and imaging.

As methotrexate is a potentially toxic drug, frequent blood counts, liver and renal function tests are required. In all cases conservative management with methotrexate resulted in undetectable serum β-hCG, a decrease in the size of partial placenta retained, and undetectable vascularity.

Conclusions
The three presented cases demonstrate that conservative treatment of persistent partial increta placenta can be successful. Although methotrexate causes a rapid involution of the placenta, there is not enough evidence to
Figure 1. — Doppler US on the 24th postpartum day showing no vascularity and a slight decrease in the placental mass (49 x 42 mm).

Figure 2. — Five months postpartum US showing that the mass measured 33 x 21 mm.

Figure 3. — Eight months following delivery a normal pelvic US can be seen.

Figure 4. — Doppler US on the 9th postpartum day showing that the placental mass was in the right horn of the uterus, invading the myometrium and measuring 33.3 x 32.8 mm.

Figure 5. — Doppler US on the 9th postpartum day showing increased vascularity.

Figure 6. — Eleven months following delivery US revealed a calcified mass of 15 x 9 mm.

Figure 7. — Doppler US on the 4th postpartum day confirmed a portion of retained placenta in the left horn, measuring 69 x 50 mm up to the serosa, with increased vascularity.

Figure 8. — MRI images showing an enlarged uterus with a portion of placental tissue, but excluding percreta placenta.

Figure 9. — Four months postpartum a US showing that the mass measured 24 x 22 mm.
suggest its routine administration in all cases of placenta accreta, especially considering its cytotoxicity. It should probably be reserved for cases of placenta percreta or when there is continuing active vascularity associated with persistent serum β-hCG levels.

Unless a life threatening hemorrhage occurs, a conservative approach is recommended even in women who do not want to preserve their fertility, considering the morbidity associated with cesarean hysterectomy. The utility of methotrexate treatment in the conservative management of placenta accreta requires further evaluation, and there is need for an agreement on a standardized protocol.

References


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Placenta percreta presenting in the first trimester and resulting in severe consumption coagulopathy and hysterectomy: a case report

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Summary

Placenta percreta complicating pregnancy in the first trimester is extremely rare, and only a few cases have been reported in the literature. A patient with risk factors for placenta percreta that presented as first trimester fetal demise, unresponsive to medical management with prostaglandin, is presented. The patient required an emergency hysterectomy to control the bleeding after uterine curettage which was complicated by severe consumption coagulopathy. This rare entity can lead to significant mortality and morbidity, particularly in the background of an increased prevalence of the disease and its associated risk factors, and the large number of spontaneous and induced abortions performed worldwide.

Key words: Placenta percreta; Early pregnancy; Placenta accreta; First trimester.

Introduction

Placenta percreta is a rare and potentially life-threatening complication of pregnancy. It is most commonly identified during the third trimester and at delivery, when difficulty with removal of the placenta is noted, usually causing massive intrapartum or postpartum hemorrhage. There are only a few reports of placenta accreta presenting in the first trimester, while placenta percreta is exceedingly rare in early pregnancy. We report a case of placenta percreta presenting in the first trimester with profuse hemorrhage following D&C for fetal demise and resulting in severe consumption coagulopathy and hysterectomy.

Case Report

A 36-year-old woman, gravida 5, para 3, presented to our department 11 weeks after her last menstrual period with mild intermittent vaginal bleeding of one week’s duration. Her obstetric history was notable for an uncomplicated vaginal delivery, followed by two low transverse cesarean sections, and an uncomplicated first trimester surgical termination of pregnancy. The first cesarean section was performed because of placental abruption at term, and it was complicated by pulmonary embolism 23 days postpartum.

Pelvic examination revealed a 12-week in size uterus, with a soft and long cervix, and a closed external cervical os. No adnexal mass or tenderness was noted. The laboratory findings were normal with a hemoglobin of 13.1 g/dl, while the urine pregnancy test was positive. Transvaginal ultrasonography showed a non-viable fetus with a crown-rump-length of 3.6 cm, consistent with fetal demise. There was no free fluid in the pouch of Douglas.

The estimated intraoperative blood loss was 1,500 ml, and intraoperative fluid replacement included 5,600 ml crystalloid, 2,000 ml colloid, seven units of packed red blood cells, and four units of fresh frozen plasma. An emergency laparotomy was carried out, keeping uterine perforation in mind. The uterus was found to be adherent to the posterior surface of the bladder, and on exposure perforation of the bladder occurred. There was no intraperitoneal bleeding nor evidence of uterine perforation. However, over the anterior lower uterine segment, a 3-cm in diameter oval purple area of subserosal myometrial hemorrhage was identified. Total abdominal hysterectomy was performed. The pathologic examination of the specimen revealed chorionic villi penetrating throughout the myometrial thickness to the serosa in both the uterine corpus and endocervix with no intervening decidua basalis layer (Figure 1).
Discussion

Reports of placenta accreta presenting in the first trimester are exceedingly rare, and a MEDLINE search from 1966 to 2007 revealed a total of only seven histologically proven first-trimester cases of placenta percreta [1-7]. The most common clinical manifestation has been severe hemorrhage precipitated by curettage for spontaneous or induced miscarriage.

Anticipating this rare but potentially catastrophic complication of pregnancy at any gestational age is of primary importance, particularly in the presence of risk factors as previous cesarean section, uterine curettage, manual removal of placenta, uterine infection, and other pregnancy-associated complications. This is imperative for proper preoperative and intraoperative planning, especially regarding the availability of blood products and early resort to hysterectomy, as well as for the proper counseling of patients. A high index of suspicion is still required for these patients, until hopefully in the future, improvements in imaging techniques will enable a more accurate preoperative diagnosis.

References

A case with diffuse uterine leiomyomatosis and review of the literature

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Summary

Leiomyomas and diffuse uterine leiomyomatosis are smooth muscle tumors of the uterus. Diffuse uterine leiomyomatosis is a benign and extremely rare condition in which the uterus is symmetrically enlarged as a result of the almost complete replacement of the myometrium by innumerable poorly defined, confluent nodules. The etiology of these neoplasms is not completely understood. Initial symptoms of the diffuse uterine leiomyomatosis usually are abdominal pain and abnormal uterine bleeding. Similar to uterine leiomyomas, patients with leiomyomatosis present with menorrhagia, dysmenorrhea, abdominal pain, infertility, and pelvic pressure. Hormonal treatment usually fails to control the symptoms, anemia, or tumor growth after treatment is stopped. As a result, despite patients being in the third or fourth decades of life, hysterectomy has been the only permanent treatment option offered to patients for treatment of the symptoms related to uterine fibroids in diffuse leiomyomatosis. A case of a patient with a huge uterine mass (2,650 g in weight) who underwent hysterectomy due to diffuse uterine leiomyomatosis is presented together with a review of the literature.

Key words: Diffuse uterine leiomyomatous; Leiomyoma; Management.

Introduction

Leiomyomas or benign fibroids are smooth muscle tumors of the uterus. They are encountered in up to 25% of women in active reproductive life. The etiology of these neoplasms is not completely understood, however, their high prevalence among premenopausal women and regression in postmenopausal years suggest an association with hormones [1].

One of the other smooth muscle tumors of the uterus is diffuse uterine leiomyomatosis. Both leiomyoma and diffuse uterine leiomyomatosis are thought to be neoplastic processes [2]. Diffuse leiomyomatosis is a rare condition in which the uterus is symmetrically enlarged as a result of the almost complete replacement of the myometrium by innumerable poorly defined, confluent nodules [3]. The previously reported cases were described as “diffuse leiomyomatosis”, “complete fibromyomatosis”, “myomatosis”, or “diffuse myomatous tendency” [1].

Leiomyomas have been studied cytogenetically and have been shown to be clonal neoplasms with consistent cytogenetic alterations. In multiple leiomyomata, the tumors have been shown to have originated from different neoplastic clones independently, rather than representing spread of the same tumor in the uterus [4, 5].

Baschinsky et al. have reported that various tumor sites within the diffuse uterine leiomyomatosis were of different clonal origin, and this supports the independent origin of the neoplastic clones. They suggested that diffuse uterine leiomyomatosis may be an exuberant example of multiple uterine leiomyomas budding into each other and blending imperceptibly to the extent that the single nodules could not be readily identified by gross examination [1]. The nodules blend with each other and merge imperceptibly with the surrounding less-cellular normal myometrium. On microscopic examination the nodules are said to be compact fascicles and interweaving bundles of benign smooth muscle cells [6].

Domnitz et al. and Grignon et al. reported cases of pregnancy in the presence of this disorder [9, 10]. The course may be complicated by cervical incompetence, spontaneous premature rupture of membranes, delivery by cesarean section, and intrapartum hemorrhage necessitating hysterectomy [10].
Case Report

A 23-year-old woman (gravida 1, para 1) was referred to our hospital because of abnormal uterine bleeding of several years duration. Her symptoms included severe hypermenorrhea, dysmenorrhea, abdominal distension, and pelvic pain. The physicians she attended previously recommended she undergo hysterectomy with the diagnosis of uterine myomatosis but the patient did not have the operation. In that time interval she had been intermittently treated with innumerable courses of hormonal therapy such as progesterone, combined oral contraceptives, GnRH analogs, and various kinds of non-steroid anti-inflammatory drugs. She had also been taking iron supplements for anemia for a long time. Uncontrolled menstrual bleeding and severe anemia were noted, and because of these her daily life was affected very badly. In addition her pelvic pain had worsened over the previous three months. Finally she and her husband decided on hysterectomy and they came to our clinic.

Pelvic ultrasound scan showed a pelvic mass. The uterus was enlarged symmetrically and had a dimension of 20 x 18 cm. There were multiple myomas with undetermined borders. We decided to perform laparotomy. In the intraoperative observation, the uterus was on the midline, 20 x 20 x 10 cm in size (Figure 1), and the color was pinkish-white. The uterus had a multinodular appearance and soft consistency, and it was symmetrically enlarged as a result of the almost complete replacement of the myometrium by innumerable but hardly defined nodules. The other pelvic organs, parametrium and ovaries were normal. Because of the very enlarged uterus and limited surgical field it was impossible to reach the cervix. First we performed a subtotal hysterectomy and then we took out the cervical portion. The uterus weighed 2,650 g postoperatively.

At pathologic examination the cervical portion was 8 x 7 x 7 cm, and the corpus was 20 x 20 x 10 cm in dimension. Section surfaces were grayish, yellowish-pink, and included multiple intramural and subserosal myomatous nodules. In the histologic sections of these nodules there were neoplastic structures containing smooth muscle fibers which were crossing each other and benign neoplastic parts which had focal hyalinization and degeneration (Figure 2).

Discussion

Diffuse uterine leiomyomatosis is a benign and extremely rare condition. In 1979 Lapan et al. claimed that diffuse leiomyomatosis of the uterus prevents myomectomy [11]. In 2000, Baschinsky et al. [1] found 14 well documented cases of diffuse uterine leiomyomatosis in the literature. Patient ages ranged from 22 to 39 years. Eleven of them were treated with total abdominal hysterectomy. The weights of the hysterectomy specimens of the patients ranged from 300 to 1200 g. In the case presented by Baschinsky et al. the hysterectomy specimen weighed 3,800 g. Kido et al. mentioned about 31 cases which had been reported till 2003 [12]. Two of these 31 cases included concomitant parametrial and pelvic or bilateral ovarian involvement [13, 14]. None of the diffuse leiomyomatosis cases recurred after hysterectomy. In subsequent years 11 more cases were added to the literature [7, 15-18]. The heaviest operation specimen in the English literature we found was the case presented by Baschinsky et al. [1].

There are few data on the metastasizing ability of uterine leiomyomatosis. Two cases of benign metastasizing leiomyomatosis that appeared in the bone and lungs have been reported [16, 17].

In a few families and isolated patients with Alport syndrome (about 5%), with proven alpha 5 chain type IV collagen (COL4A5) gene mutation, an association with leiomyomatosis of the esophagus, tracheobronchial tree and female genitals has been reported [19].

It is necessary to differentiate leiomyomatosis microscopically from diffuse uterine adenomyosis and diffuse endometrial hypertrophy. These two entities could be rejected by initial histologic examination [13].

The differential diagnosis of leiomyomatosis includes multiple leiomyomas, intravascular leiomyomatosis and endometrial stromal sarcoma [1, 3, 6, 7].

Leiomyomatosis can be distinguished from leiomyoma due to the uniform symmetrical involvement of the entire
myometrium by smooth muscle nodules without distinct borders between the nodules, whereas cases of multiple leiomyomas tend to have asymmetrical involvement of the uterus and sharp circumscription of the individual leiomyomas [1, 7].

Intravascular leiomyomatosis has a creamy to yellowish color, and there are intravascular extensions of wormlike smooth muscle tumor with multinodular irregular or indistinct margins. Intravascular leiomyomatosis can be distinguished histologically from diffuse leiomyomatosis by the presence of some or all of the neoplastic smooth muscle within the vascular channels.

Endometrial stromal sarcoma is characterized by its invasive growth having an abrupt transition with the normal myometrium, and it has a sheetlike, rather than fascicular, growth pattern. In contrast to diffuse leiomyomatosis, small neoplastic cells with round to oblong nuclei and scant cytoplasm separate the thick walled vessels. In addition, endometrial growth and intravascular growth are usually present [7].

Nisolle et al. have reported that estrogen receptors (ERs) and progesterone receptors (PRs) were significantly higher in leiomyoma than in the adjacent myometrium [20]. Kim SJ et al. found that the ER level was equal in both normal myometrium and leiomyomatosis areas but PR level was higher in leiomyomatosis tissue, so they suggested that diffuse leiomyomatosis lesions might be under the influence of progesterone, which might play a major role in their growth. They proposed an antiprogestin agent as a treatment alternative for diffuse uterine leiomyomatosis according to their results [7]. However hormonal treatment usually fails to control the symptoms, anemia, or tumor growth after treatment is stopped [8].

As a result, despite patients being in the third or fourth decades of life, hysterectomy has been the only permanent treatment option offered for treatment of the symptoms related to uterine fibroids in diffuse leiomyomatosis [6].

Aki Kido et al. presented a case of diffuse leiomyomatosis treated by uterine arterial embolization (UAE) as an alternative to hysterectomy for the treatment of leiomyomas and reported that UAE successfully controlled the symptoms and reduced uterine volume with no major complications in their patient [12]. Fedele et al. treated only medically with GnRH analogues (GnRH-a) one of three premenopausal women with diffuse uterine leiomyomatosis associated with persistent menorrhagia. The patient conceived spontaneously as soon as medical treatment was discontinued; at 34 gestational weeks, an emergency cesarean section followed by hysterectomy was performed for vaginal bleeding and a healthy 2,400 g baby was born. In the other two cases they performed an “extreme” myomectomy, including the removal of a large portion of corporal myometrium. Regular menses were restored in these two patients: one had no pregnancy desire and the other was not able to conceive after two IVF-ETs in 2004 [15].

Women with early-stage diffuse uterine leiomyomatosis can be treated by hysteroscopic resection, which has the benefit of successfully preserving the uterus. Yen et al. described five cases of successful hysteroscopic resection for early-stage diffuse uterine leiomyomatosis. The uterus was successfully preserved and a normal amount of menstruation was restored in all (5/5) patients. All (3/3) patients who wished to conceive had successful conceptions, with four healthy deliveries [18].

In the presented case, the patient had two aunts who had undergone operations for uterine myoma in her family history. Clinical presentation, symptoms and family history of our case was consistent with the literature. In comparison with most cases reported in the literature, our case had a large uterus that weighed 2,650 g. We think that medical treatment failure was due to the extremely enlarged uterus. Surgery was in August 2003 and there has been no any recurrence in four years of follow-up.

In the case of medical treatment failure, the symptoms of the patient may be resolved with surgical resection of the nodules by protecting the normal anatomic structure of the uterus, and even pregnancy may be possible. But in huge masses associated with diffuse uterine leiomyomatosis if protecting the normal anatomic structure is not possible, hysterectomy may be a treatment modality after the patient’s approval.

References


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Successful treatment of advanced endometriosis with extremely high CA 125 and moderately elevated CA 15-3 levels

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Introduction

Endometriosis is a benign condition that usually presents with chronic pelvic pain and infertility. CA 125 is a tumor marker which is particularly used for monitoring of epithelial ovarian cancers, but which is also elevated in advanced endometriosis cases. CA 15-3 is another tumor marker mostly related with breast cancer. Extremely elevated levels of CA 125 are well documented in endometriosis, however, elevated levels of CA 125 and CA 15-3 are not so common in advanced endometriosis. The case was successfully treated with laparoscopy and combined low-dose oral contraceptive with one year of follow-up. To the best of our knowledge among the reported cases this is the highest CA 15-3 level ever reported with an extremely elevated CA 125 level.

Case Report

A 29-year-old woman, gravida 1, para 1, was admitted to our clinic with persistent pelvic pain. She had a history of dysmenorrhea for several years. She had regular periods and her last menstrual cycle was seven days earlier. Transvaginal ultrasound (US) revealed bilateral ovarian endometrioma (right 5 x 4 cm and left 4 x 3 cm in size). CA 125 level was 2345 U/ml (upper reference limit < 35 U/ml) and CA 15-3 level was 100.8 U/ml (upper reference limit < 29 U/ml). CA 19-9 was 0.6 U/ml (upper reference limit < 37). To rule out malignancy, magnetic resonance imaging (MRI) and breast US were performed. Pelvic MRI also revealed bilateral ovarian endometriomas whereas breast US was normal.

Laparoscopy showed that both ovaries with endometrioma cysts were located in the Douglas pouch. All pelvic peritoneal surfaces were found to be covered with diffuse endometriotic implants. Peritoneal washings were collected, laparoscopic excision of endometriotic cysts while preserving both ovaries was performed, and peritoneal biopsies were taken. Histopathological diagnosis was bilateral ovarian endometrioma and endometriotic foci. CA 125 and CA 15-3 were returned to reference ranges dramatically two weeks after laparoscopy. Continuous low-dose combined oral contraceptive was used for further endometriosis suppression for six months. After one year of follow-up the patient did not express any symptoms of pelvic pain and transvaginal US demonstrated normal ovaries with normal anatomical locations.

Discussion

Endometriosis is known as an extrauterine location of endometrial glands and stroma due to different mechanisms like retrograde menstruation and implantation [1], coelomic metaplasia [2] or vascular transport [3]. Regardless of any mechanism, ectopic localization of endometrial tissue might result in pelvic organ damage which is usually presented with pelvic pain and infertility.

CA 125 is a tumor antigen that is expressed from coelomic epithelium and its derivatives such as endometrial cells. CA 125 is particularly used as a tumor marker for epithelial ovarian cancers, as well as advanced carcinomas of the endometrium and endocervix. CA 125 can be elevated during pregnancy, menstruation, adenomyosis, leiomyomas, and pelvic inflammatory disease. Elevated levels of CA 125 were also reported in advanced endometriosis. Extremely high levels of CA 125 were reported both due to acute rupture of endometrioma and unruptured endometrioma cases (9300 U/ml and 7900 U/ml, respectively) [4, 5].

CA 15-3 is a tumor antigen particularly used to monitor breast cancer. CA 15-3 is a glycosylated transmembrane molecule which is produced by glandular epithelial cells and also present in endometrial glands. CA 15-3 levels were found to be the same throughout the ovarian cycle [6]. Elevated levels of CA 15-3 in endometriosis had been reported before [7, 8], but the levels were around normal values and we could not detect any CA 15-3 level as high as we have reported.

Summary

We present the case of a patient with advanced endometriosis who presented with chronic pelvic pain, bilateral unruptured ovarian endometrioma, massive peritoneal implants and extremely elevated CA 125, and also elevated CA 15-3 levels. Laparoscopy revealed bilateral unruptured ovarian endometrioma and diffuse peritoneal endometriotic implants. Increased association of elevated levels of CA 125 and CA 15-3 is not so common in advanced endometriosis. The case was successfully treated with laparoscopy and combined low-dose oral contraceptive with one year of follow-up. To the best of our knowledge among the reported cases this is the highest CA 15-3 level ever reported with an extremely elevated CA 125 level.

Key words: Endometriosis, CA 125, CA 15-3, Laparoscopy.
CA 19-9 is another tumor antigen that can be elevated in endometriosis. Elevated levels of CA 125, CA 19-9 and CA 15-3 together, CA 125 and CA 19-9 together, CA 19-9 and CA 15-3 alone were also reported in endometriosis (9). One other study demonstrated high levels of CA 125, CA 19-9 and CA 15-3 in the peritoneal fluids of endometriosis cases (10). In all these cases serum levels of CA 15-3 did not pass the upper reference limit; only in the peritoneal fluid study were the values higher.

In the present case the question is; in the state of unruptured ovarian endometrioma the elevated levels of CA 125 and CA 15-3 out of the menstrual phase should result from endometrioma cysts, endometriotic foci or both? The answer should be ovarian endometrioma because in a two-week period both tumor markers returned to normal levels that it was not possible to destroy all the diffuse endometriotic foci during laparoscopy or within the low dose combined oral contraceptive that had been started after the operation. The reason why CA 19-9 levels did not change was unclear. Further in vivo and in vitro studies are needed to understand the exact association between tumor markers and endometriosis.

We have reported a case of advanced endometriosis with extremely high CA 125 and moderately elevated CA 15-3 levels. After ruling out malignancy, instead of high levels of tumor antigens by MRI or during the laparoscopy, taking peritoneal washings, laparoscopic excision of endometriomas and collecting peritoneal biopsies would be the choice of surgery. In cases of advanced endometriosis with massive peritoneal implants suppression therapy with low-dose combined oral contraceptive in a continuous fashion up to 6-12 months should be the choice of medical management [7]. To the best of our knowledge we have reported the highest CA 15-3 level with an extremely high CA 125 level in advanced endometriosis out of the menstrual period.

References


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